PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER

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Dedicated To
My Respected
Teachers, Family
&Friends

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Dr. Deepika Bansal

LIST OF ABBREVIATIONS

AOR	Adjusted Odds Ratio
BDNF	Brain-derived neurotrophic factor
BL	Bifidobacterium longum
CNS	Central nervous system
CTRI	Clinical Trials Registry- India
DASS	Depression Anxiety And Stress Scale
GABA	Gamma-Aminobutyric Acid
HAMD	Hamilton Rating Scale For Depression
HPA	Hypothalamic-Pituitary- Adrenal Axis
H/O	History of
IQR	Inter Quartile Range
LHFM	Lactobacillus helveticus-fermented milk
LR	Lactobacillus rhamnosus
MADRS	Montgomery-Asberg Depression Rating Scale
MAO I	Monoamine Oxidase Inhibitors
MDD	Major Depressive Disorder
M.I.N.I.	Mini-International Neuropsychiatric Interview
NMDA	N-Methyl-D-Aspartate
SD	Standard Deviation
SSRI	Selective Serotonin Reuptake Inhibitors
SNRI	Serotonin And Norepinephrine Reuptake Inhibitors
TCA	Tricyclic Antidepressant

ABSTRACT

INTRODUCTION*:* Major Depressive Disorder (MDD) is a burdensome disorder. It is now being considered that inflammation, oxidative stress, dysregulated function of the hypothalamic-pituitary-adrenal axis and decreased neurotrophins including brain-derived neurotrophic factor are the major pathophysiological pathways that play a role in pathogenesis of MDD [2]. Probiotics have the potential to modulate the gut microbiome [13].

OBJECTIVE: To study the effect of probiotic supplementation on symptoms of depression in patients of major depressive disorder already on treatment with antidepressants.

MATERIALS AND METHODS*:* This was a randomized, double blind, placebo controlled trial conducted on 100 patients of MDD presenting to DMC&H who were already on treatment with antidepressants. Patients were allotted to consecutively receive either probiotic or the placebo for 6 weeks. Both the patient and researcher were blinded. Patients were screened using MINI scale having only MDD and severity of depression was assessed at 0,3 & 6 weeks each using HAM-D and MADRS scales.

RESULTS: After 6 weeks of intervention, patients who received probiotic supplements had significantly decreased HAM-D score with mean±SD score 28.04 ± 4.03 at Week 0 to 18.04 ± 3.55 at Week 6, as compared to placebo group with a mean score 28.00 ± 4.83 at Week 0 to 20.78 ± 8.12 at Week 6. MADRS score in probiotic group decreased from mean score 41.34 ± 4.56 at Week 0 to 28.40 ± 3.93 at Week 6, as compared to placebo group with a mean score 41.56 ± 7.05 at the Week 0 to 30.22 ± 10.60 at the Week 6.

CONCLUSION: These results suggested that adding probiotic to ongoing antidepressant treatment may have positive effect on treatment of depression in patients with MDD. Large scale trials can be conducted according to the results of the current study.

KEYWORDS: Gut-Brain Axis, Major Depressive Disorder, Gut Microbiome, Probiotics.

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INTRODUCTION

Major Depressive Disorder (MDD) is a chronic psychiatric illness which significantly affects quality of life of an individual. Point prevalence for major depressive disorder is 12.9 percent, and its lifetime prevalence is 10.8 percent [1]. Depression has major social, personal and occupational consequences and thus, greatly lowers the quality of life of an individual. It also leads to suicidal thoughts and ideations potentially leading to death. It usually affects women more than men with mean age of onset around 40 yrs. According to the Diagnostic and Statistical Manual of Mental Disorders (5th ed.) [2], diagnostic criteria of MDD includes: “the presence of sad, empty, or irritable mood which is characterized by loss of interest in routine activities and is often accompanied by feelings of guilt, hopelessness, and changes in appetite and sleep, as well as somatic and cognitive changes which can significantly affect the individual’s personal, social and occupational capacity to function” (American Psychiatric Association, 2013) [3].

Depression- An Inflammatory Disease

Earlier, it was generally accepted among clinicians and scientists that MDD was a disorder of reduced monoaminergic activity in the brain. Consequently, most pharmacological treatments currently available were solely targeting these neurotransmitters such as dopamine, serotonin, norepinephrine and their receptors. Others have also postulated glutamate receptor involvement (e.g. with the use of the N-methyl-D-aspartic acid [NMDA] receptor agonist ketamine) or the modulation of cholinergic and γ-aminobutyric acid (GABA) transmission in the etiopathogenesis of depression. Those treatments which target these neurotransmitters, lessen the symptoms of depression by directly modulating neurotransmitter function in the brain. The pathogenesis of MDD is much more complicated, rather than just correcting the imbalance of certain neurotransmitters in the brain. It also includes other systems such as the immune system and its role in neuroinflammation which have been implicated in several mental

illnesses including MDD [4]. Thus, modulators of host immunity are currently being researched as novel treatment targets for mental illness[5].

Inflammation, oxidative stress, dysregulated function of the hypothalamic-pituitary-adrenal (HPA) axis, and decreased neurotrophins including brain-derived neurotrophic factor (BDNF) are the major pathophysiological pathways that play a role in pathogenesis of MDD [6]. Gut Brain axis is involved in the regulation of the functions of the Central Nervous System (CNS) and mood. Therefore, it has been implied in the pathogenesis of neuropsychiatric disorders [7]. There occurs elevations of pro-inflammatory cytokines including interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-a), and C-reactive protein (CRP) in Major Depressive Disorder. There is evidence of stress-related physiology, such as elevated cortisol levels in the development of depression. Brain-related biomarkers of depression include lowered levels of dopamine, serotonin, Gamma-Aminobutyric Acid (GABA), and various neurotrophic factors such as brain-derived neurotrophic factor (BDNF). Thus, depression is correlated to inflammation, stress, immune dysfunction, and a hyperactive hypothalamus-pituitary-adrenal (HPA) axis [3].

The Gut-Brain Axis and Gut Microbiome – Bacteria in Our Gut and Their Importance to Mental Health

A complex bidirectional communication between the gut and the brain has been found, which has attracted lot of interest in psychiatric research. The mechanisms underlying the gut–brain axis include the gastrointestinal tract, central nervous system (CNS), autonomic nervous system, enteric nervous system, neuroendocrine system and immune system [8].

When a microbial community inhabits a certain habitat, it is called microbiota. Every bacterium contains its own genetic material. The totality of all genes and genomes found in the microbiota is referred to

as “microbiome”. The composition of the gut microbiome differs with age, physical characteristics, geographical origin, disease, dietary patterns and environmental factors. There is a bidirectional communication pathway between the intestine and the brain called the “gut-brain axis”, which connects the enteric nervous system to the central nervous system. Thus, the intestinal microbiota and diet play an important role in this gut-brain interaction system, which has shown to be involved in the pathogenesis of various psychiatric disorders such as depression and anxiety. For instance, a reduction in the number of microbial species has been reported in a range of mental disorders. Thus, it is recommended that gut microbiota intervene directly in our neurotransmitter metabolism, especially in the serotonin metabolism.

In preclinical murine studies, it was shown that probiotics based on changes in serotonin metabolism have an antidepressant and anxiolytic effect. In particular, Lactobacillus plantarum has shown antidepressant efficacy in mouse models. Secondly, a damaged intestinal flora (“dysbiosis”) contributes to an increased permeability of the intestinal mucosa (“leaky gut”), which leads to an increased immune response and chronic neuroinflammation which is a major cause of mental illness. This inflammatory response could in turn stimulate cytokine production in our body, which occurs when bacterial components (such as lipopolysaccharides from the bacterial cell wall) bind on circulating macrophages and monocytes in bloodstream. Cytokines and soluble intercellular signaling molecules are generated in the brain and in the periphery and are involved in the pathophysiology of psychiatric disorders such as depression and anxiety disorders as they influence neurotransmitter synthesis, release and reuptake. Dietary approaches could alter gut bacteria and gut bacterial metabolites such as short chain fatty acids, decrease cytokine production which in turn lower overall inflammation and enhance the effect of current psychopharmacological approaches [9].

From Probiotics to Psychobiotics

Probiotics are a preparation of microbes in sufficient numbers, which can change the microflora in the host which results in advantageous health effects to host [10]. Probiotics have been prescribed for many gut-related disorders, but as the understanding of the gut-brain axis has evolved, a new era of probiotics referred to as “psychobiotics” for their influence on the CNS, cognition, and behavior [3]. Thus, it is now established that patients with major depression have a pro-inflammatory phenotype with increased levels of pro-inflammatory cytokines. Elevations in plasma interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF alpha) are those most efficaciously demonstrated. Certain probiotic bacteria have an anti-inflammatory property with the capacity to alter a pro-inflammatory phenotype [11]. It is postulated that the effects of probiotics on psychiatric symptoms are mediated through the gut-brain axis by reducing systemic inflammation and modulating neurotransmission. By maintaining the integrity of the GI lining, probiotics decrease permeability caused by compromised tight junctions of endothelial cells in the GI lining, thus preventing bacteria and toxins to leak from the GI tract into the bloodstream. Lipopolysaccharides, an endotoxin which is present in the membrane of gram-negative bacteria, induces an immune response including a release of proinflammatory cytokines, which is a common phenotype seen in depressed patients. These proinflammatory cytokines can make their way to other organs such as the brain and therefore affects the neurotransmission by interfering with metabolic pathways and thus leads to depressive symptoms such as low mood, fatiguability, sleep disturbances and reduced appetite [5].

Evidence now suggests that the bacterial commensals of the gut can influence psychiatric conditions such as depression, schizophrenia and autism [12]. It is now understood that depression can stem from biological, social and environmental factors and therefore, the present research seeks to address possible biological and environmental origins of depression [3].

Inspite of the noteworthy advancement in the understanding of MDD, existing understanding of MDD is still incomplete, treatment options are insufficient and therefore newer understanding into MDD pathogenesis is required. Therefore, one novel area of investigation related to MDD pathophysiology is the gut microbiome and its relation to brain [13].

This proposal seeks to build on prior research to produce an inclusive nutritional intervention that potentially will have a more significant impact on treatment of depressive disorders. This study focuses on the use of probiotics as an adjuvant to the already existing treatments in expectation of creating a protocol that will yield a more significant reduction on depressive symptoms [3]. This study is therefore, a step to the understanding of the effect of therapeutic modulation of gut microbiota using specific strains of probiotics on brain neurotransmitter functioning [14].

AIMS AND OBJECTIVES

To study the effect of probiotic supplementation on symptoms of depression in patients of major depressive disorder already on antidepressants.

REVIEW OF LITERATURE

Akkasheh et al [15] conducted a randomized, double-blind, placebo-controlled clinical trial which included 40 patients with a diagnosis of MDD based on DSM-IV criteria whose age ranged between 20 and 55 years. Patients were randomly allocated into two groups to receive either probiotic supplements (n=20) or placebo (n=20) for 8 weeks. Probiotic capsule consisted of three viable and freeze-dried strains: Lactobacillus acidophilus (2x10^9 CFU/g), Lactobacillus casei (2x10^9 CFU/g), and Bifidobacterium bifidum (2x10^9 CFU/g). There was no significant difference between the two groups in terms of the dietary intake of study participants. It was observed that after 8 weeks of intervention, patients who received probiotic supplements had significantly decreased Beck Depression Inventory total scores (p=0.001) compared with the placebo.

Kazemi et al [16] conducted a randomized, double blind, placebo-controlled clinical trial on one hundred and ten depressed patients who were randomly assigned to receive the probiotic (Lactobacillus helveticus and Bifidobacterium longum), prebiotic (galacto-oligosaccharide) or placebo for 8 weeks. It was observed that a total of 81 subjects completed the trial (out of which 28 participants were in the probiotic group, 27 participants were in the prebiotic group and 26 participants were in the placebo group). It was observed that after 8 weeks of intervention, a significant decrease in BDI score with the probiotic supplementation (17.39±9.1) was seen as compared to the placebo (18.18±15.55) and prebiotic supplementation (19.72±14.14) (p=0.042). Overall an improvement in BDI score compared with placebo with no significant effect of prebiotic supplementation was seen after 8 weeks of probiotic supplementation to subjects with MDD.

Steenbergen et al [17] reported a randomized triple-blind, placebo-controlled trial. It was a pre and post-intervention assessment design in healthy participants without any current mood disorder symptoms. In this trial, 20 participants received a 4-week probiotic food-supplement

intervention, while 20 control participants received placebo for 4 weeks. Revised Leiden index of depression sensitivity scale was used to assess the Cognitive reactivity to sad mood. It was observed that the participants who were assigned the probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood, after 4 weeks intervention, as compared to the participants who were assigned the placebo intervention. Thus, it was concluded that the intake of probiotics help decrease the negative thoughts and sad mood.

Messoudi et al [4] conducted a double-blind, placebo-controlled, randomized clinical trial for 30 days duration and had 55 volunteers in the study. The assessment of the participants was done using Hospital Anxiety and Depression Scale (HADS), Perceived Stress Scale along with Hopkins Symptom Checklist (HSCL-90) and Coping Checklist (CCL) and 24 hour urinary free cortisol (UFC). It was noted that daily administration of probiotic formulation significantly reduced psychological distress in volunteers, as measured by the HSCL-90 scale (global severity index, $p<0{\cdot}05$; somatisation, $p<0{\cdot}05$; depression, $p<0{\cdot}05$; and anger hostility, $p<0{\cdot}05$), HADS scale (HADS global score, $p<0{\cdot}05$; and HADSanxiety scale, $p<0{\cdot}06$), and CCL (problem solving, $p<0{\cdot}05$) and the UFC level ($p<0{\cdot}05$). Thus, it was demonstrated that probiotics had beneficial psychological effects in healthy human volunteers.

Bruce-Keller et al [18] summarized influence of gut microbiome over physiological function, with focus on neurobehavioral and neurological impairment which is based on ever growing understanding of the gut-brain axis. This study concluded that microbiota, food, bile acids, and mucosal factors interact with each other in intestinal lumen. The intestinal mucosa then propagates the information to other organs such as the brain via blood circulation and sensory nerves. This affects the mucosa and eventually the gut microbiota directly or indirectly (via other organs) through changes in autonomic nervous

system (ANS) and endocrine outflow and through changes in food intake quantity and choice.

Flowers and Ellingrod [19] verified that a symbiotic bidirectional communicative relationship exists between the host and the gut microbiome in their study. It emphasized on the relationship of bacteria present in our gut to cognitive and emotional processes. Thus, alteration in this relationship is important in mental health practices. It was observed that illnesses such as major depressive disorder are more seen in patients with gastrointestinal disorders such as inflammatory bowel disease, whose pathology has been strongly linked to microbiome function.

Mayer [20] explained that the interaction between the gut and the brain plays an essential role in the mood state of an individual. The interest in this gut–brain axis shows a complex, bidirectional communication relationship. It emphasizes that along with the proper maintenance of gastrointestinal system and digestion, the gut microbiome is likely to have significant impact on affect, motivation and higher cognitive functions.

Dinan et al [21] reviewed the mechanism of action of psychobiotics. The authors explained that psychosocial stressis one of the prominent causal factors in the pathogenesis of major depression. The hypothalamic-pituitary adrenal axis is activated by stress which causes the release of corticotropin releasing hormone (CRH) and vasopressin (AVP) along with gut dysbiosis. It further, causes the passage of lipopolysaccharide (LPS) and other molecules into the bloodstream and results in development of a proinflammatory phenotype in individuals suffering from depression. This is characterized by high interleukin-1 (IL-1), interleukin-6 (IL-6) levels, and prostaglandin E2 (PGE2). Conventional antidepressants act directly on monoamines and regulate their synthesis. However, psychobiotics increase the production of anti-inflammatory cytokine interleukin 10 (IL-10), which results in decrease of proinflammatory cytokines, and suppresses the

hypothalamic-pituitary-adrenal axis activity.

Cenit et al [22] reviewed the role of the gut microbiota in neuropsychiatric disorders and the underlying mechanisms which contributes to mental health. Various communication pathways between gut and brain have been identified which includes immune, endocrine and neural pathways. Thus, the microbiota plays a role in mental health by regulating inflammatory and endocrine secretions. It also results in synthesis of neuro active compounds through interventions with probiotics in animal models.

Osadchiy et al [23] reviewed the clinical literature on brain gut microbiome interactions which states that there exists a well-characterized bidirectional communication channel, between the gut and the brain. It involves neural, endocrine, and inflammatory mechanisms. It also stated that brain gut microbiome interactions are programmed during the first 3 years of life, including the prenatal period however, gut brain axis can be modulated by diet, medications, and stress throughout life. They proposed that the brain gut microbiome axis has been recognized as the potential targets for novel drug development for psychiatric illnesses.

Pinto-Sanchez et al [24] conducted a randomized, double-blind, placebo-controlled study. It included 44 adults with Inflammatory Bowel Disease (IBS) and diarrhea and mild to moderate anxiety and/or depression (based on the Hospital Anxiety and Depression scale). Patients were then randomly assigned to two groups where one group received probiotic BL (Bifidobacterium longum) and other received placebo for 6 weeks. At week 0, 6 patient's levels of anxiety and depression, Inflammatory Bowel Disease (IBS) symptoms, quality of life, and somatization were determined using validated questionnaires. It showed that at the end of week 6, majority of patients in the probiotic group had reduction in depression scores as compared to patients in the placebo group ($p=0.04$).

Miyaoka et al [25] conducted a study to evaluate the efficacy and safety of Clostridium butyricum MIYAIRI 588 (CBM588) in combination with antidepressants in adult patients diagnosed with Treatment resistant depression. It was an 8-week open-label study which included 40 patients who were on antidepressant treatment. Patients were randomized to receive an add on treatment with either CBM588 (n=20) or control (n=20). Results of the study stated that the addition of Clostridium butyricum MIYAIRI 588 (CBM588) (60 mg/d) along with ongoing antidepressant treatment provided significant improvement in depression.

Ghorbani et al [26] conducted a randomized, double blind trial. The trial was conducted on 40 patients who had been suffering from moderate depression. All patients were started on fluoxetine (20 mg/d) for 4 weeks. Then, patients were randomized to either receive a synbiotic capsule or placebo along with fluoxetine for 6 weeks. The study demonstrated that there was a greater improvement in HAM-D score in synbiotic treated patients (Mean±SD = -19.25±1.71) as compared to group receiving placebo (Mean±SD = -17.75±2.05; p=0.024).

Liu et al [27] conducted a random-effects meta-analysis of 34 controlled clinical trials. These trials evaluated the effects of prebiotics and probiotics on depression and anxiety. It showed that prebiotics did not differ from placebo for depression or anxiety, however, probiotics resulted in significant effects for depression ($p<0.01$) and anxiety ($p=0.03$).

Kim et al [28] reviewed the bidirectional relationship between the gut and the brain. Gut and brain were connected through neural, immune-mediated, and metabolic mechanisms. It stated that there was increased inflammation, activation of the HPA axis, and altered levels of neurotransmitters as a result of gut dysbiosis. Reduced integrity of the gastrointestinal barrier leads to bacterial migration (“leaky gut”) and inflammation in brain. Probiotics play a potential role to normalize and reduce such inflammation.

Zhou et al [29] in his review supported that the gut microbiota is able to bidirectionally communicate with the brain which is known as the gut–brain axis. It also stated that in the absence or dysfunction of normal gut flora, psychiatric manifestations can occur.

Mayer et al [30] explored the role of the gut microbiota in modulating the brain. This study stated that the role of the gut-brain axis and stated that the gut microbiota influences the emotional behavior, stress- and pain-modulation systems, and brain neurotransmitter systems. Additionally, it also stated that, microbiota changes by the use of probiotics and antibiotics exert modulatory effects on these measures in adults.

Latalova et al [31] in their study stated that stress response, memory functions, social behavior and mood are modulated by gut microbiota. Furthermore, it emphasized on the fact that microbiota play a role in the development of the central nervous system which suggested the role of microbiota in human psychiatric disorders. Microbiota affects phenomena that are involved in pathogenesis of depression, such as the exaggerated response to stress and inflammatory theory of depression. It stated that the ingestion of probiotics have antidepressant effects.

Rhee et al [32] summarized the evidence in support of the existence of bidirectional interactions between the gut and brain. It concluded that this bidirectional relationship between brain and enteric microbeshelps in modulating gut function and thus, involved in the modulation of emotions, pain perception and general well-being.

Liang et al [33] stated that many factors, including stress, antibiotics, and unhealthy diet, can induce the microbiota disturbance which could result in mental illnesses. Thus, improving the gut microbiota through intake of probiotics, prebiotics, and healthy diet promotes mental health. It will not only help patients suffering from mental disorders but also promote cognition and resilience in healthy people.

Valentina Ignatova [34] stated that probiotics improve carbohydrate malabsorption and have beneficial effects on the immune system. These mechanisms are associated with decrease in symptoms of depression.

Grace Lucas [35] in his review study explained the concept of 'embodied mental health'. It explains that mental health is not a separate entity from physical health and explores the entanglements of mental health within a horizontal bio-psychosocial framework.

Reus et al [36] in their review showed that there is role of inflammation and microglial activation in the pathogenesis of psychiatric disorders and thus supports the role of probiotics in depression.

Logan et al [37] strongly advocated the role of probiotics in lowering systemic inflammatory cytokines, decrease oxidative stress and improve nutritional status. The effect of probiotics on systemic inflammatory cytokines and oxidative stress will ultimately lead to increased brain derived neurotrophic factor (BDNF) which plays a role in pathophysiology of depression.

Mohamaddi et al [38] conducted a randomized double-blind trial on 70 petrochemical workers. Here, the subjects were randomly divided into three groups. First group was to receive 100 g/day probiotic yogurt along with one placebo capsule (n=25), second group received one probiotic capsule daily and 100 g/day conventional yogurt (n=25) and third group received 100 g/day conventional yogurt and one placebo capsule (n=20) for 6 weeks. Results showed that a significant improvement of General Health Questionnaire (GHQ) was observed in the probiotic yogurt (p=0.007) as well l as in the group receiving probiotic capsule (p=0.001). Also, a significant improvement in Depression Anxiety Stress Scale (DASS) scores in the probiotic yogurt (p=0.02) and the group receiving probiotic capsule (p=0.006). However, it was seen that there was no significant improvement in the conventional yogurt group (p=0.05 for GHQ and p=0.08 for DASS).

The consumption of probiotic yogurt or probiotic capsule was assessed to have beneficial effects on mental health parameters.

Foster et al [39] explained the relation between stress and microbiota. It also focused on how alterations in microbiota will influence stress-related behaviors. It was stated that anxietyand depressive symptoms are a result of alterations in microbiota. This modulates plasticity related, serotonergic, and GABAergic signaling systems in the brain. Thus, it helps in developing new treatment approaches to prevent and treat psychiatric disorders.

Dash et al [40] suggested the role of the gut microbiota in influencing behaviour and mood. The gut microbiota interplays with environmental risk factors which includes poor dietary habits and stress. Thus, gut microbiota is a new target for the prevention and treatment of depression.

Marx et al [41] discussed the neurobiological mechanisms such as inflammation, oxidative stress, the gut microbiome, epigenetic modifications and neuroplasticity which are likely modulated by dietary habits. Thus, dietary interventions can provide significant clinical benefit. Furthermore, probiotics as well as other nutraceuticals such as omega-3 fatty acids, folate, S-adenosylmethionine and N-acetyl cysteine are under research.

Dinan & Cryan [42] summarized the theoretical explanation of how microbiota plays a role in depression. Their study demonstrated that modulation of brain-gut axis functionis associated with specific alterations in the stress response and overall behavior. Major depressionis a common, debilitating stress-related disorder where by patients frequently have hypothalamic-pituitary-adrenal (HPA) alterations such as elevated cortisollevels in plasma, elevated corticotropin releasing factor (CRF) levels in the cerebrospinal fluid coupled with a failure to suppress cortisol in response to dexamethasone challenge. Moreover, marked increases in the

concentrations of pro-inflammatory cytokines are also common biological hallmark of the disease. Microbes can influence the functioning of the HPA and immune system and thus, the modulation of gut bacteria can help in management of psychiatric disorders.

Rios et al [43] emphasized and studied the gut brain axis. It also provided evidence of the link between gastrointestinal microbiota and mood disorders. It also elaborated on the benefits of using probiotics in the treatment of MDD.

Jacka et al [44] conducted a 'SMILES' trial. It was a 12-week, parallel-group, single blind, randomized controlled trial which included an adjunctive dietary intervention along with already ongoing treatment, in the treatment of moderate to severe depression. The participants were allocated into 3 groups, one group using psychotherapy and pharmacotherapy combined; second group was using exclusively psychotherapy; and third group was using only pharmacotherapy. It was seen that there was greater improvement on the MADRS scores in the dietary support group than the social support control group, $p < 0.001$. Thus, dietary intervention helps in management of psychiatric disorders because of the beneficial effects of probiotics.

Bambling et al [45] conducted a cohort study (n=12). The study was conducted on SSRI-resistant MDD patients and they were assessed using Beck Depression Inventory (BDI), Outcome Questionnaire 45 (OQ45) and Quality of Life (QoL). At the end of study, it was concluded that probiotics and magnesium orotate when added as adjuvant in MDD treatment significantly improved MDD symptoms.

Benton et al [46] conducted a double-blind placebo-controlled trial on general population for a 3-week period. They were randomized to either receive a probiotic containing milk drink, or a placebo. The results of the study showed that there was improvement in mood after the consumption of probiotic-containing yoghurt as compared to those whose mood was initially poor.

LV et al [47] reviewed the literature exploring the targeting of gut brain axis as adjuvant treatment to existing options as microbiota play a role in the pathogenesis of schizophrenia and major depressive disorder.

Evrensel et al [48] in their systematic meta analysis suggested the influence of gut microbiota on mood. They concluded that by using healthy diet and probiotics, the gut microbiota can be regulated which has important benefits for preventing and treating depression. The understanding of gut-brain axis could aid in treatment of neuropsychiatric disorders.

Sherwin et al [49] focused on the role for gut-brain axis in modulating mood symptoms. The bacteria of our gut communicate with the brain through neurotransmission, neurogenesis and microglia activation. Dysregulation of the gut microbiota leads to development of psychiatric disorders. Thus, probiotics are being proposed for potential therapeutic interventions.

A systematic review by Fond et al [50] studied the association between development of major psychiatric disorders and microbiota dysbiosis. The study was done to explore potential therapeutic opportunities which target the microbiota dysbiosis in psychiatric patients.

Chung et al [51] investigated the effects of Lactobacillus helveticus-fermented milk (LHFM), on cognitive function in healthy adults. They conducted a 12-week, double-blind, randomized controlled study. It was seen that after the administration of LHFM for 12 weeks, an improvement on cognitive tests was seen as compared to the placebo group.

Gruenwald et al [52] conducted a 6-month study. He evaluated the role of a probiotic multivitamin preparation in persons suffering from stress. An overall 40.7% improvement in stress was noted, at the end of the study. Also, it was seen that there was a decrease of 29% in the frequency of infections and of 91% in gastrointestinal discomforts. Thus, it was concluded that this probiotic multivitamin is suitable for

reducing stress as well as for improving the immune system.

A study by Marcos et al [53] aimed to evaluate the effect of milk fermented with yogurt cultures and Lactobacillus casei on subjects who had academic stress. They concluded that there was no significant treatment effect between both the groups.

Hilimire et al [54] conducted a study to assess social anxiety symptoms. It showed that there were fewer social anxiety symptoms in persons who consumed fermented foods which are likely to contain probiotics.

Rao et al [55] conducted a study on patients of Chronic Fatigue Syndrome. They were randomized to receive either Lactobacillus casei or a placebo daily for two months. It was found that there was a significant decrease in anxiety symptoms among those taking the probiotic as compared to controls ($p=0.01$).

Seigel et al [56] studied the efficacy of B. longum in a double-blind study design. Participants were randomly assigned to either receive placebo or probiotic capsules. The results of the study showed no significant reduction in symptoms between groups.

Romijn et al [57] conducted a double blind study where participants were randomly allocated to receive either a probiotic preparation (containing Lactobacillus helveticus and Bifidobacterium longum) or a placebo for 8 weeks. No significant difference was seen between the probiotic and placebo groups.

Ostlund-Lagerstrom et al [58] conducted a double-blinded, randomized, placebo-controlled clinical trial. This study was done to evaluate the effect of the probiotic strain Lactobacillus reuteri on digestive health and wellbeing. There were no significant effects seen at the end of the study.

Kouchaki et al [62] conducted a study. Their study showed that as compared to the placebo, probiotic intake improved beck depression

inventory (p<0.001), general health 35 questionnaire (p<0.001) and depression anxiety and stress scale (p=0.001).

In a study conducted by Marotta et al [63] 38 healthy volunteers assigned to an experimental or control group consumed a daily dose of a probiotic mixture (containing Lactobacillus fermentum LF16, L. rhamnosus LR06, L. plantarum LP01, and Bifidobacterium longum BL04) or placebo, respectively, for 6 weeks. The results showed a significant improvement in mood was observed in the experimental group, with a reduction in depressive mood state, anger, and fatigue, and an improvement in sleep quality.

In a study conducted by Chahwan et al [64], 71 participants were randomly allocated to either probiotic or placebo, which was consumed daily over eight weeks. Participants in the probiotic group demonstrated a significantly greater reduction in cognitive reactivity compared with the placebo group which is a marker of depression.

In a study conducted by Slykerman et al [65] the results showed that the participants in the probiotic treatment group Lactobacillus rhamnosus HN001 (HN001), reported significantly lower depression scores and anxiety scores than those in the placebo group, (p=0·002).

In specific probiotic studies where individual strains were studied, a study conducted by Majeed et al [66] showed significant change (p=0.01) in favour of the B. coagulans MTCC 5856 for the primary efficacy measured on Hamilton Rating Scale for Depression (HAM-D) and Montgomery-Asberg Depression Rating Scale (MADRS).

To summarize, as per the existing literature, a bidirectional relationship exists between the gut and the brain through neural, immune-mediated, and metabolic mechanisms. Thus, gut dysbiosis leads to increased inflammation, as well as, activation of the HPA axis, and altered levels of neurotransmitters which in turn, leads to development of psychiatric manifestations. The stress induced inflammation of the gastrointestinal barrier causes the migration of bacteria also known as

“leaky gut”. Probiotics have the potential to normalize and reduce such inflammation. As there is dearth of Indian data on this concern, our study planned to find out the role of probiotic supplementation in addition to the already antidepressant treatment in patients of MDD in a tertiary care setting.

MATERIALS AND METHODS

SOURCE OF DATA:

The study was conducted on recently diagnosed 100 patients in age group of 18-65 years who were already on treatment for depression for a period of 6-12 weeks, diagnosed as Major Depressive Disorder presenting to Department of Psychiatry in DMC&H, Ludhiana.

METHOD OF COLLECTION OF DATA

SAMPLE COLLECTION: The study was conducted on 100 patients of Major Depressive Disorder in age group of 18-65 years presenting to Department of Psychiatry in DMC&H, Ludhiana who were already on treatment with antidepressants.

INCLUSION CRITERIA:

1. The patients with a diagnosis of Major Depressive Disorder according to DSM-5 criteria presenting to Department of Psychiatry in DMC&H, Ludhiana.
2. Age group between 18-65 years.
3. Patients who have agreed upon no changes in their routine physical activity and dietary intake throughout the study.

EXCLUSION CRITERIA:

1. Any other major psychiatric disorder in patients of Major Depressive Disorder.
2. Regular intake of probiotics during last 2 months before recruitment for the study.
3. History of any renal, hepatic, cardiovascular or respiratory disease in the patient.
4. Intake of antioxidant or omega 3 supplements less than 6weeks before beginning of study.
5. Female patients currently having pregnancy and lactation.
6. Any type of substance abuse in the patient.

7. Patients with history of heart attack and stroke following a specific diet.
8. Patients with intake of antibiotics 2 weeks before the study or during study.
9. Immunocompromised patients.
10. Patients who have not given consent for the study.

METHODOLOGY:

The study was a randomized, double blind, placebo controlled trial, conducted on 100 patients of Major Depressive Disorderin age group of 18-65 years, presenting to Department of Psychiatry in DMC&H, Ludhiana who were already on treatment with antidepressants. Patients were allotted to consecutively receive either probiotic or the placebo for 6 weeks where both the patient and researcher were blinded. Patients were screened using M.I.N.I. having only MDD and severity of depression was assessed at 0,3 & 6 weeks, each using HAM-D and MADRS scales. Antidepressant treatment was continued and the Probiotic as well as the Placebo were administered as an add on treatment. Unblinding was done after the study was completed.

TOOLS:

1. SOCIO-DEMOGRAPHIC PROFORMA
2. MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW (M.I.N.I.) [59]
3. HAMILTON RATING SCALE FOR DEPRESSION (HAM-D) [60]
4. MONTGOMERY ASBERG DEPRESSION RATING SCALE (MADRS) [61]

1) SOCIO- DEMOGRAPHIC PROFORMA was applied on the patient.

2) MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW [59]- MINI questionnaire for the assessment of the psychiatric co-morbidities. The Mini-International Neuropsychiatric Interview (M.I.N.I.) version 5.0.0 is a short structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and Europe, for psychiatric disorders. It is divided into 16 modules-

identified by letters, each corresponding to a diagnostic category. With an administration time of approximately 15 minutes, it was designed to meet the need for a short but accurate structured psychiatric interview for multi-centre clinical trials.

3) **HAMILTON RATING SCALE FOR DEPRESSION (HAM-D) [60] -** It is a multiple item questionnaire used to provide an indication of depression, and as a guide to evaluate recovery. Max Hamilton originally published the scale in 1960 and revised it in 1966, 1967, 1969, and 1980. The questionnaire is designed for adults and is used to rate the severity of their depression by probing mood, feelings of guilt, suicide ideation, insomnia, agitation or retardation, anxiety, sedation and somatic symptoms. HAM-D is a 21 multiple choice questionnaire which doctors may use to rate the severity of depression in a patient. Each question has 3-5 possible responses which increase in severity.

Scoring: The interpretation of scale is as follows-

- **0-9**- normal
- **10-13**- mild depression
- **14-17**- moderate depression
- **>17**- severe depression

4) **MONTGOMERY-ASBERG DEPRESSION RATING SCALE [61] :** The Montgomery- Asberg Depression Rating scale is a 10 item diagnostic questionnaire used to measure the severity of depressive episodes in patients with mood disorders. It was designed in 1979 by British and Swedish researchers. The items are rated on a continuum of 0-6.The questionnaire includes questions on following symptoms – Apparent sadness, Reported sadness, Inner tension, Reduced sleep, Reduced appetite, Concentration difficulties, Lassitude, Inability to feel, Pessimistic thoughts, Suicidal thoughts.

Scoring: The interpretation of scale is as follows-

- **0-6**- Normal/ Symptoms absent
- **7-19**- Mild Depression

- **20-34**- Moderate Depression
- **>34**- Severe Depression

STATISTIC ALANALYSIS:

Data will be described in terms of range; mean ± standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables will be done using Student t-test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and exact test will be used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

TRIAL REGISTRATION:

Clinical Trials Registry- India: CTRI/2020/05/025027 [Registered on: 05/05/2020]

OBSERVATIONS AND RESULTS

The study was a double blind randomized controlled trial, conducted on 100 patients of Major Depressive Disorder presenting to DMC&H who were already on treatment with antidepressants. Patients were allotted to consecutively receive either probiotic or the placebo for 6 weeks where both the patient and researcher were blinded.

Unblinding was done after the study was completed which revealed that Group A is Placebo and Group B is the Probiotic capsule containing probiotic not less than 2.5 billion cells of *Lactobacillus acidophilus* (R 0052), *Lactobacillus rhamnosus* (R 0011), *Bifidobacterium longum* (R 00175), *Bacillus coagulans* (SNZ 1969), and *Saccharomyces boulardii*.

TABLES AND FIGURES

Table 1: Distribution of the Patients in Terms of Group (n=100)

Group	Number of patients	Percentage (%)
A	50	50.0
B	50	50.0
Total	100	100

Table 1 shows 50 patients were in Group A and 50 patients were in Group B

Table 2: Distribution of the Patients in Terms of Age (n=100)

Age	Number of patients			Fisher's Exact Test	
	Group A	Group B	Total	χ2	P Value
18-25 Years	5	2	7 (7.0%)	4.086	0.264
26-40 Years	18	27	45 (45.0%)		
41-55 Years	21	15	36 (36.0%)		
56-65 Years	6	6	12 (12.0%)		
Total	50	50	100 (100.0%)		

Table 2 shows that there was no significant difference between the two groups in terms of distribution of Age (p=0.264).

Figure 1: Distribution of the Patients in Terms of Age (n=100)

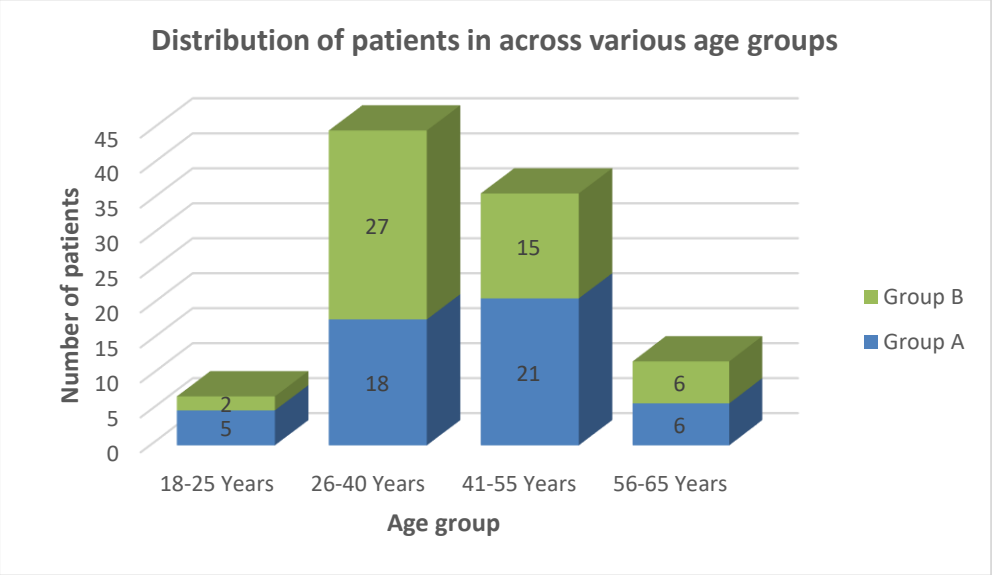

Figure 1 shows that majority of the patients in Group A (42%) were in 41-55 years age group and in Group B (54%) were in 26-40 years age group.

Table 3: Distribution of the Patients in Terms of Gender (n=100)

Gender	Number of patients			Chi-Squared Test	
	Group A	Group B	Total	χ2	P Value
Male	28	28	56 (56.0%)	0.000	1.000
Female	22	22	44 (44.0%)		
Total	50	50	100 (100.0%)		

Table 3 shows that there was no significant difference between the two groups in terms of distribution of gender (χ2=0.000, p=1.000).

Figure 2: Distribution of the Patients in Terms of Gender (n=100)

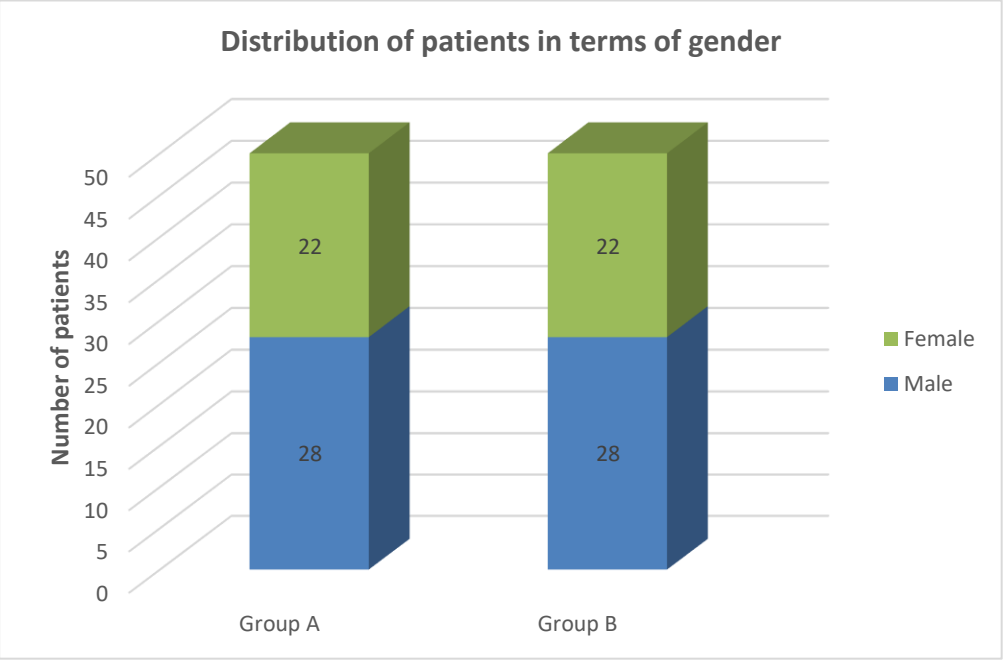

Figure 2 shows that majority of the patients in both Group A and B (56%) were males.

Table 4: Distribution of the Patients in Terms of Education (n=100)

Education	Number of patients			Fisher's Exact Test	
	Group A	Group B	Total	χ2	P Value
Illiterate	10	13	23 (23.0%)	4.327	0.660
Primary School	13	10	23 (23.0%)		
Middle School	5	7	12 (12.0%)		
High School	9	8	17 (17.0%)		
Higher Secondary/Diploma	6	8	14 (14.0%)		
Graduate	5	1	6 (6.0%)		
Honors/Post-Graduate	2	3	5 (5.0%)		
Total	50	50	100 (100.0%)		

Table 4 shows that there was no significant difference between the two groups in terms of distribution of education (p=0.660).

Figure 3: Distribution of the Patients in Terms of Education (n=100)

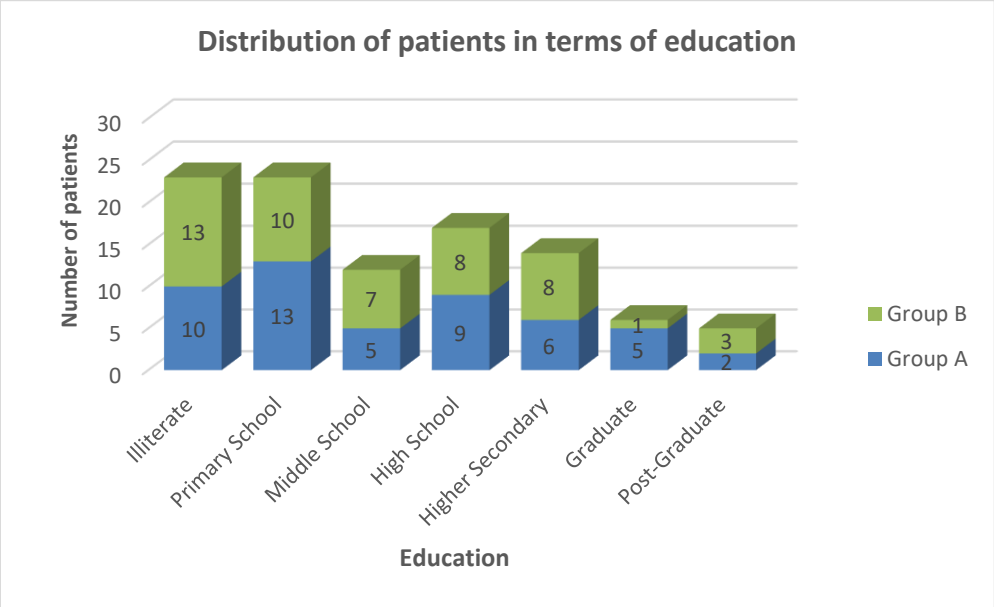

Figure 3 shows that majority of the patients in Group A (26%) had studied up to primary school and in Group B (26%) were illiterate.

Table 5: Distribution of the Patients in Terms of Occupation (n=100)

Occupation	Number of patients			Fisher's Exact Test	
	Group A	Group B	Total	χ2	P Value
Farmer	15	12	27 (27.0%)	3.439	0.885
Homemaker	13	14	27 (27.0%)		
Semi-Skilled	9	7	16 (16.0%)		
Businessman/Self-Employed	5	9	14 (14.0%)		
Skilled	4	3	7 (7.0%)		
Student	3	2	5 (5.0%)		
Unemployed	1	2	3 (3.0%)		
Retired	0	1	1 (1.0%)		
Total	50	50	100 (100.0%)		

Table 5 shows that there was no significant difference between the two groups in terms of distribution of occupation (p=0.885).

Figure 4: Distribution of the Patients in Terms of Occupation (n=100)

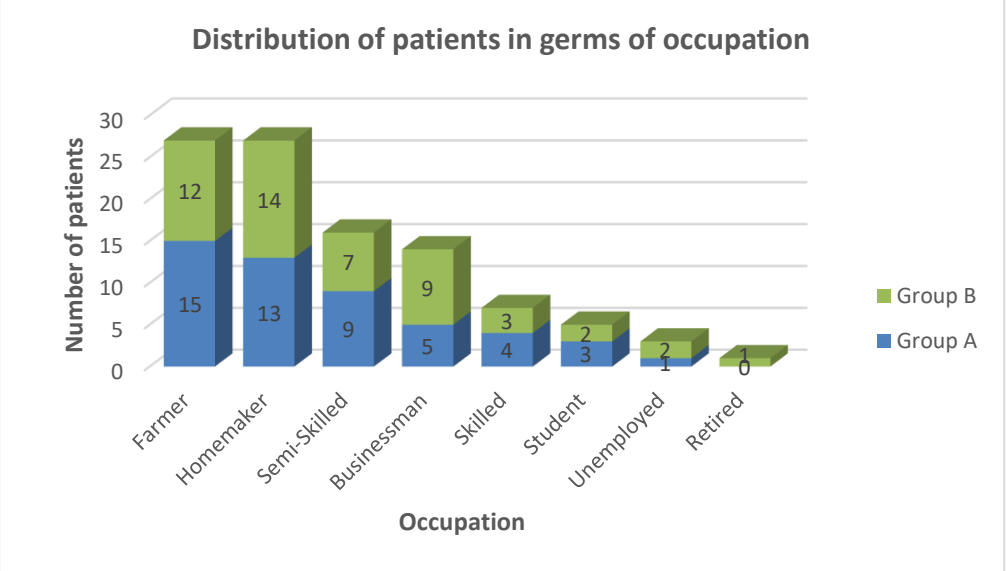

Figure 4 shows that majority of the patients in Group A (30%) were farmers and majority of the patients in Group B (28%) were homemaker.

Table 6: Distribution of the Patients in Terms of Monthly Income (Rs) (n=100)

Monthly Income (Rs)	Number of patients			Fisher's Exact Test	
	Group A	Group B	Total	χ2	P Value
Up to 10,000	22	22	44 (44.0%)	1.506	0.840
10,000-20,000	14	12	26 (26.0%)		
20,000-30,000	8	7	15 (15.0%)		
30,000-50,000	2	5	7 (7.0%)		
>50,000	4	4	8 (8.0%)		
Total	50	50	100 (100.0%)		

Table 6 shows that there was no significant difference between the two groups in terms of distribution of monthly income (Rs) (p=0.840).

Figure 5: Distribution of the Patients in Terms of Monthly Income (Rs) (n=100)

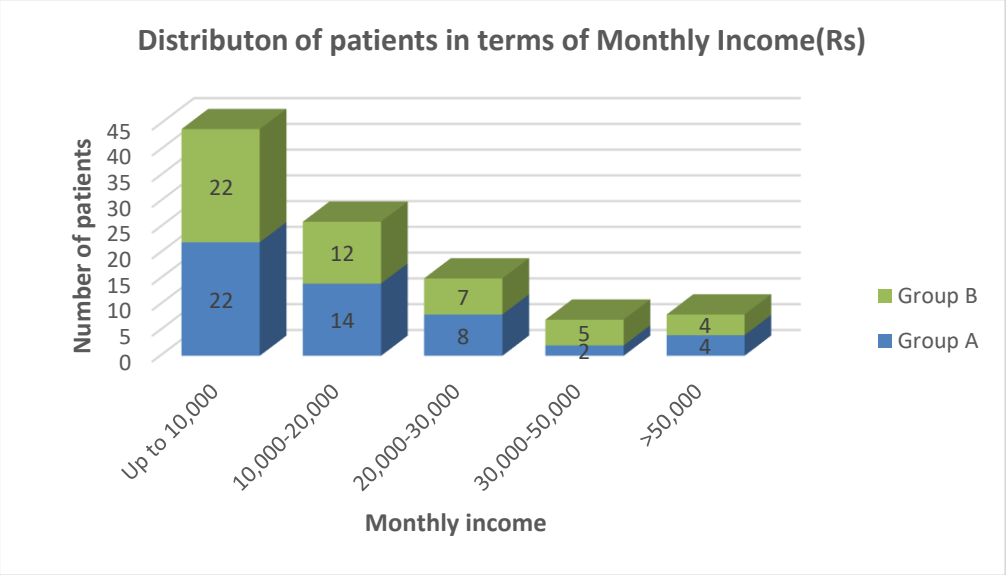

Figure 5 shows that majority of the patients in both Group A and B (44% each) had monthly income of less than Rs.10, 000.

Table 7: Distribution of the Patients in Terms of Family Type (n=100)

Family Type	Number of patients			Chi-Squared Test	
	Group A	Group B	Total	χ2	P Value
Nuclear	7	6	13 (13.0%)	0.180	0.914
Extended	15	14	29 (29.0%)		
Joint	28	30	58 (58.0%)		
Total	50	50	100 (100.0%)		

Table 7 shows that there was no significant difference between the two groups in terms of distribution of family type (χ2=0.180, p=0.914).

Figure 6: Distribution of the Patients in Terms of Family Type (n=100)

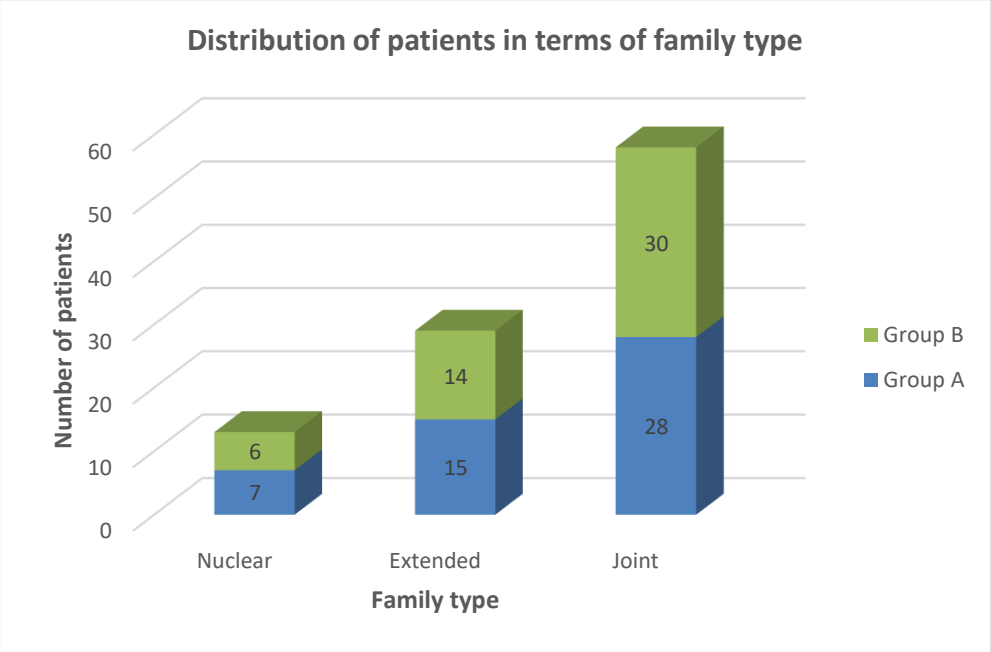

Figure 6 shows that majority of the patients in Group A (56%) and Group B (60%) belonged to joint family.

Table 8: Distribution of the Patients in Terms of Marital Status (n=100)

Marital Status	Number of patients			Fisher's Exact Test	
	Group A	Group B	Total	χ2	P Value
Married	33	32	65 (65.0%)	2.149	0.635
Unmarried	16	14	30 (30.0%)		
Divorced	1	3	4 (4.0%)		
Remarried	0	1	1 (1.0%)		
Total	50	50	100 (100.0%)		

Table 8 shows that there was no significant difference between the two groups in terms of distribution of marital status (p=0.635).

Figure 7: Distribution of the Patients in Terms of Marital Status (n=100)

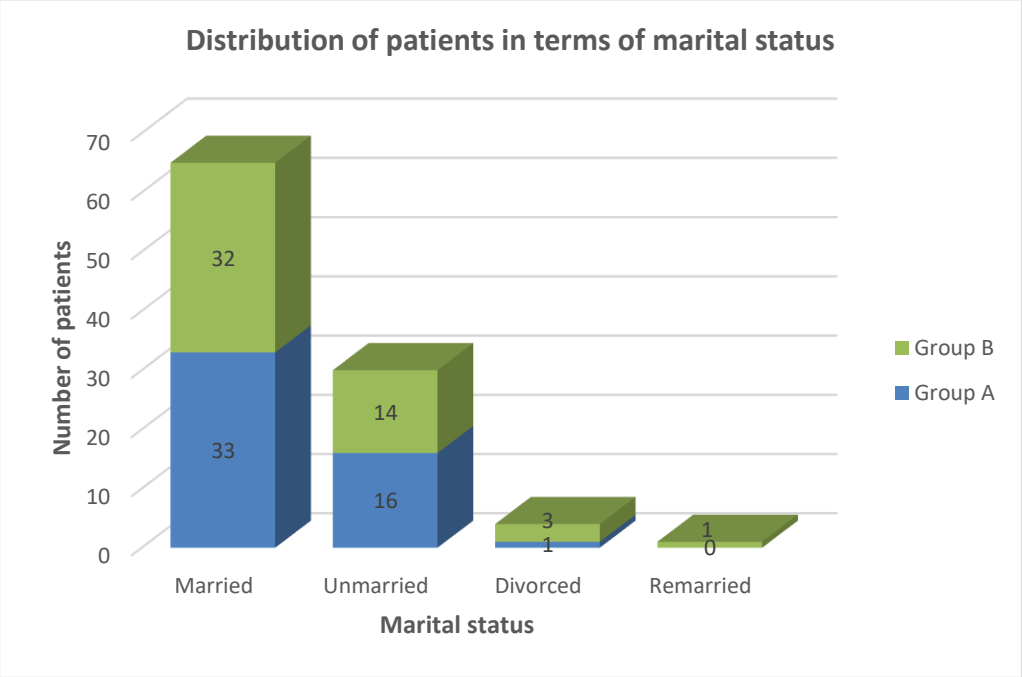

Figure 7 shows that majority of the patients in Group A (66%) and Group B (64%) were married.

Table 9: Distribution of the Patients in Terms of Family H/O Psychiatric Illness (n=100)

Family H/O Psychiatric Illness	Number of patients			Chi-Squared Test	
	Group A	Group B	Total	χ2	P Value
Yes	25	23	48 (48.0%)	0.160	0.689
No	25	27	52 (52.0%)		
Total	50	50	100 (100.0%)		

Table 9 shows that there was no significant difference between the two groups in terms of distribution of family H/O psychiatric illness (χ2=0.160, p=0.689).

Figure 8: Distribution of the Patients in Terms of Family H/O Psychiatric Illness (n=100)

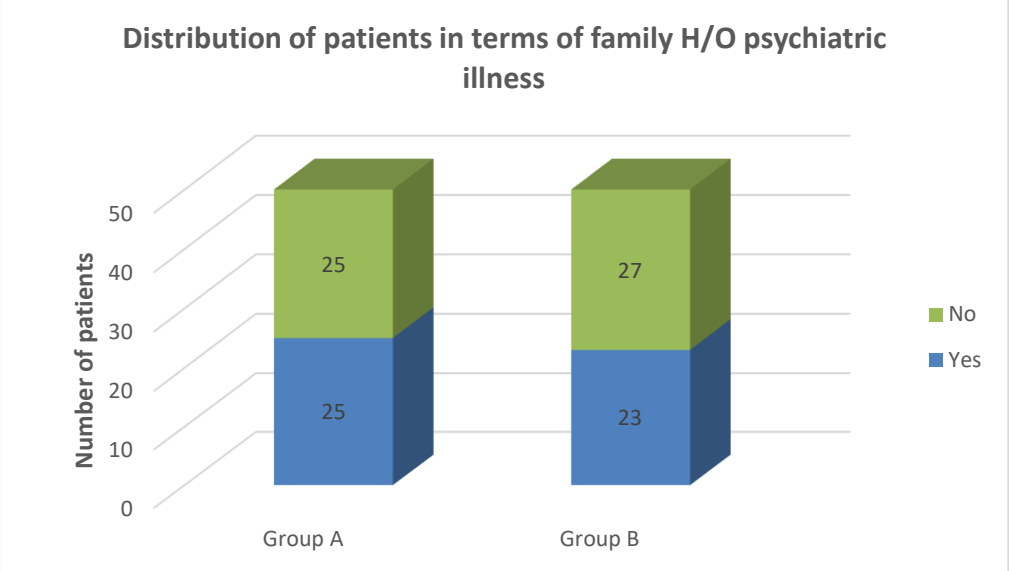

Figure 8 shows that in Group A 50% patients had Family H/O Psychiatric Illness and in Group B, majority of patients (52%) had no Family H/O any Psychiatric Illness.

Table 10: Distribution of the Patients in Terms of H/O Self-Harm or Suicidal Ideation (n=100)

H/O Self-Harm or suicidal Ideation	Number of patients			Chi-Squared Test	
	Group A	Group B	Total	χ2	P Value
Yes	33	34	67 (67.0%)	0.045	0.832
No	17	16	33 (33.0%)		
Total	50	50	100 (100.0%)		

Table 10 shows that there was no significant difference between the two groups in terms of distribution of H/O self-harm or suicidal ideation (χ2=0.045, p=0.832).

Figure 9: Distribution of the Patients in Terms of H/O Self-Harm or Suicidal Ideation (n=100)

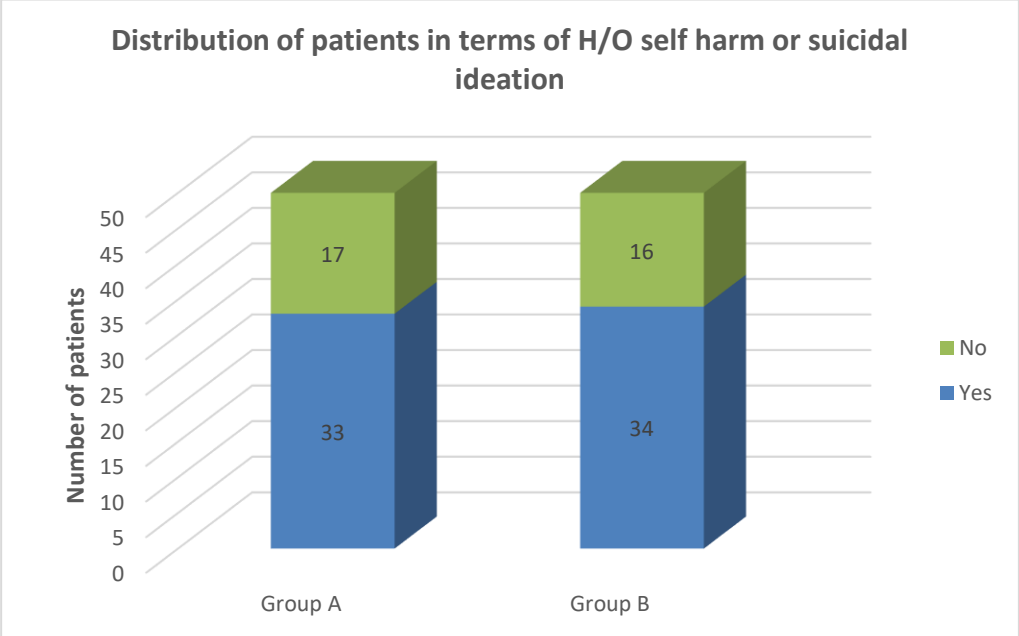

Figure 9 shows that majority of the patients in Group A (66%) and Group B (68%) had H/O Self-Harm or Suicidal Ideation

Table 11: Distribution of the Patients in Terms of Compliance to treatment (Week 6) (n=100)

Compliance to Treatment (Week 6)	Number of patients			Fisher's Exact Test	
	Group A	Group B	Total	χ2	P Value
Yes	49	48	97 (97.0%)	0.344	1.000
No	1	2	3 (3.0%)		
Total	50	50	100 (100.0%)		

Table 11 shows that there was no significant difference between the two groups in terms of distribution of compliance to treatment (Week 6) (χ2=0.344, p=1.000).

Figure 10: Distribution of the Patients in Terms of Compliance to treatment (Week 6) (n=100)

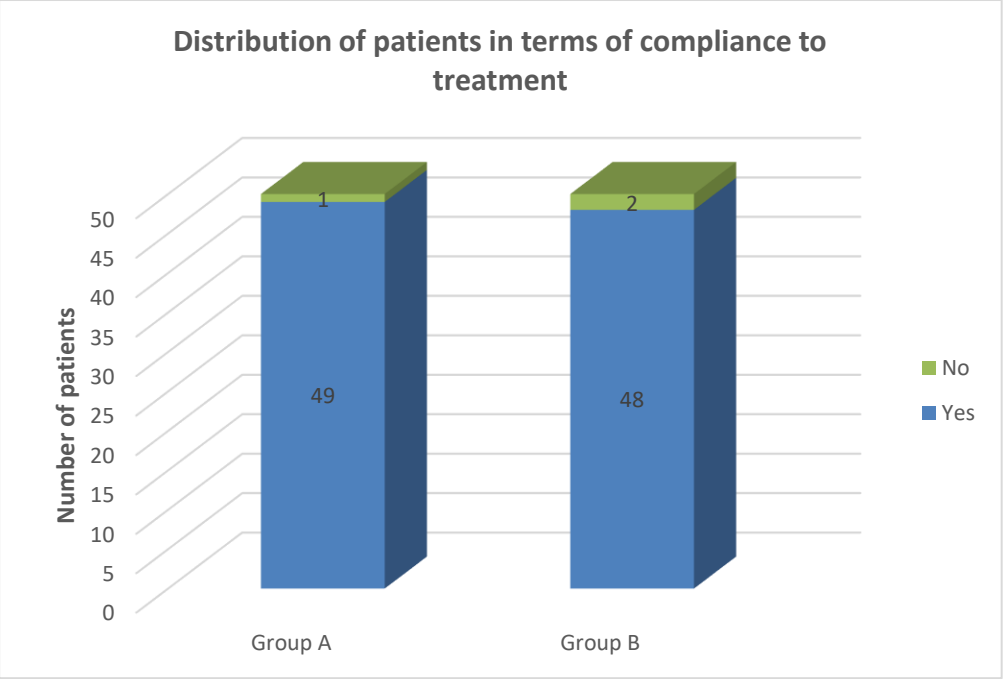

Figure 10 shows that 98% patients in Group A and 96% patients in Group B were compliant to treatment at the end of 6 weeks.

Table 12: Distribution of the Patients in Terms of Class of Antidepressant (n = 100)

Class Of Antidepressant	Number of patients			Fisher's Exact Test	
	Group A	Group B	Total	χ2	P Value
SNRI	3	3	6 (6.0%)	0.000	1.000
SSRI	47	47	94 (94.0%)		
Total	50	50	100 (100.0%)		

Table 12 shows that there was no significant difference between the two groups in terms of distribution of class of antidepressant (χ2=0.000, p=1.000).

Figure 11: Distribution of the Patients in Terms of Class of Antidepressant (n=100)

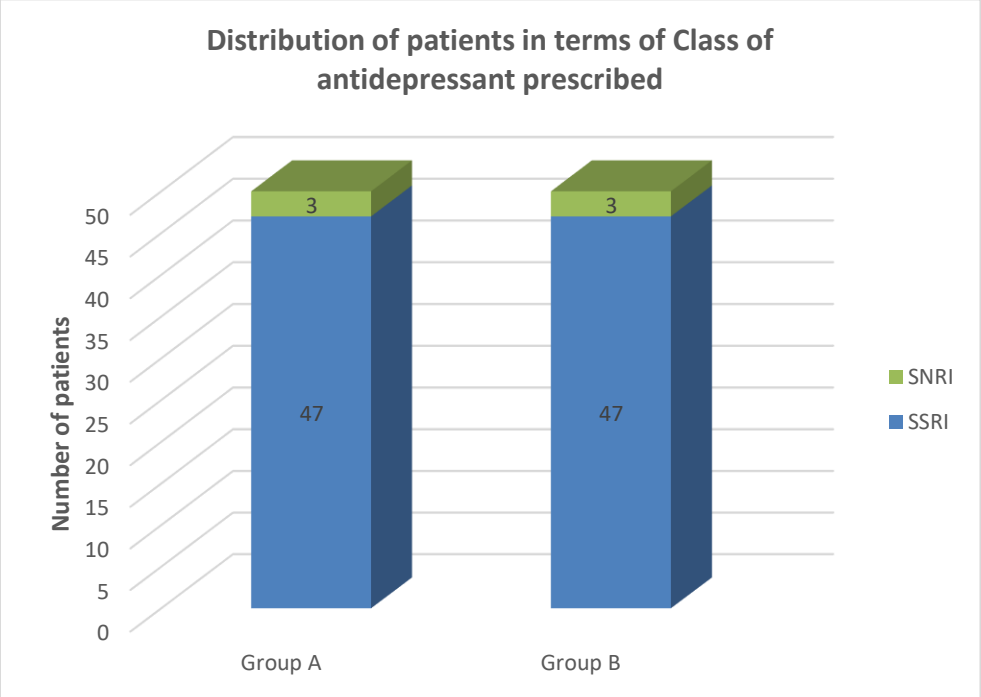

Figure 11 shows that majority of patients (94%) in both Group A and B were prescribed SSRI antidepressant and 6% were prescribed SNRI antidepressant.

Table 13: Comparison of mean, median and range of HAM-D scores at week 0, week 3 and week 6 (n=100)

HAM-D	Week 0	Week 3	Week 6
Mean (SD)	28.02 (4.42)	22.55 (5.23)	19.42 (6.40)
Median (IQR)	29 (26-30.25)	23 (19.75-26.25)	19 (17-24)
Range	16 - 38	9 - 32	4 - 31

Table 13 shows that mean HAM-D scores decreased from 28.02 at week 0 to 19.42 at week 6.

Table 14: Comparison of Group A & B in Terms of HAM-D (Week 0) (n=100)

HAM-D (Week 0)	Group		Wilcoxon-Mann-Whitney U Test	
	A	B	W	p value
Mean (SD)	28.00 (4.83)	28.04 (4.03)	1260.500	0.945
Median (IQR)	29 (26-31)	29 (27-30)		
Range	16 - 38	17 - 34		

Table 14 shows that mean HAM-D score at baseline (Week 0) is 28.00 in Group A and 28.04 in Group B and the change was insignificant (p=0.945).

Figure 12: Comparison of Group A & B in Terms of HAM-D (Week 0) (n=100)

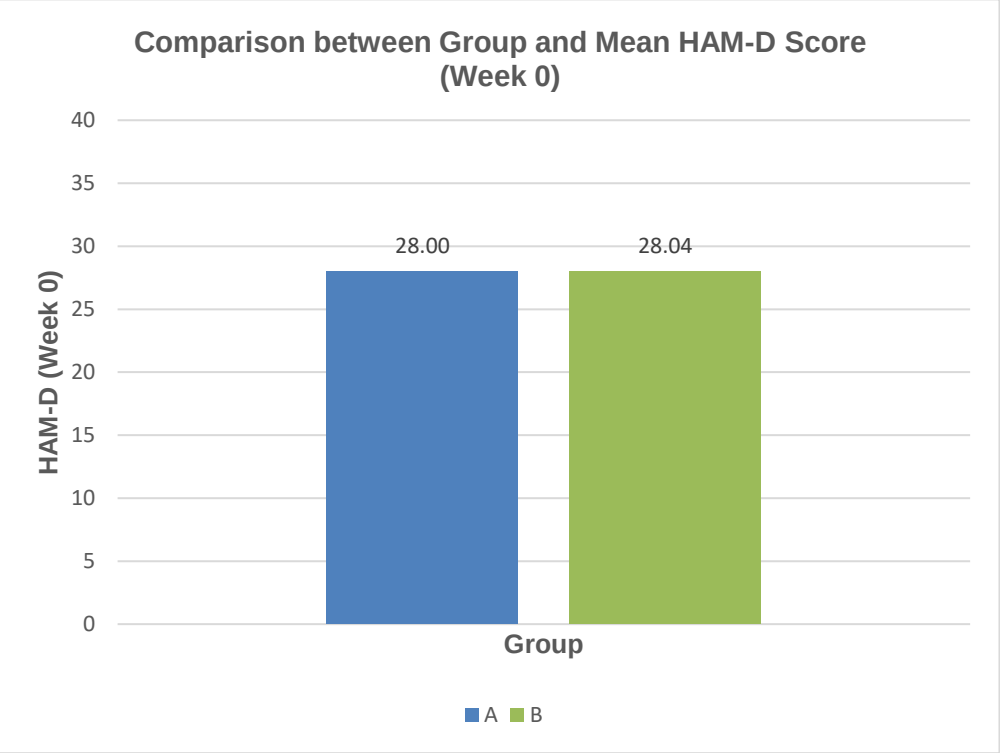

Figure 12 shows that mean HAM-D score at baseline (Week 0) is 28.00 in Group A and 28.04 in Group B and the change was insignificant (p=0.945).

Table 15: Comparison of Group A & B in Terms of HAM-D (Week 3) (n=100)

HAM-D (Week 3)	Group		Wilcoxon-Mann-Whitney U Test	
	A	B	W	p value
Mean (SD)	23.50 (6.22)	21.60 (3.84)	1699.000	0.002
Median (IQR)	26 (22.25-27)	22 (19-24)		
Range	9 - 32	12 - 30		

Table 15 shows that mean HAM-D score at Week 3 is 23.50 in Group A and 21.60 in Group B and the change was significant (p=0.002).

Figure 13: Comparison of Group A & B in Terms of HAM-D (Week 3) (n=100)

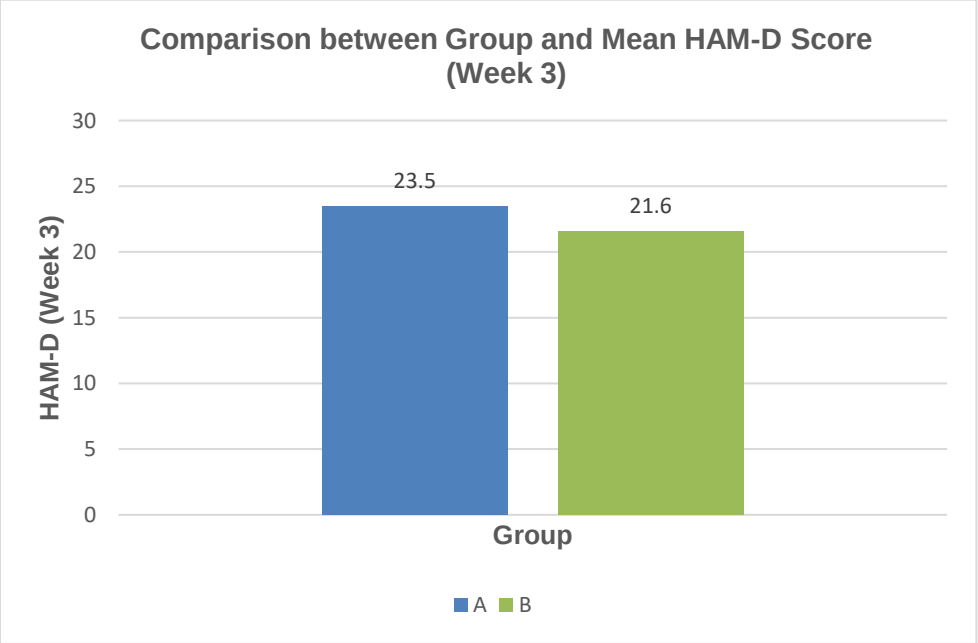

Figure 13 shows that mean HAM-D score at Week 3 is 23.50 in Group A and 21.60 in Group B and the change was significant (p=0.002).

Table 16: Comparison of Group A & B in Terms of HAM-D (Week 6) (n=97)

HAM-D (Week 6)	Group		Wilcoxon-Mann-Whitney U Test	
	A	B	W	p value
Mean (SD)	20.78 (8.12)	18.04 (3.55)	1741.000	<0.001
Median (IQR)	24 (21-26)	18 (16-19)		
Range	4 - 31	10 - 28		

Table 16 shows that mean HAM-D score at Week 6 is 20.78 in Group A and 18.04 in Group B and the change was significant (p<0.001).

Figure 14: Comparison of Group A & B in Terms of HAM-D (Week 6) (n=97)

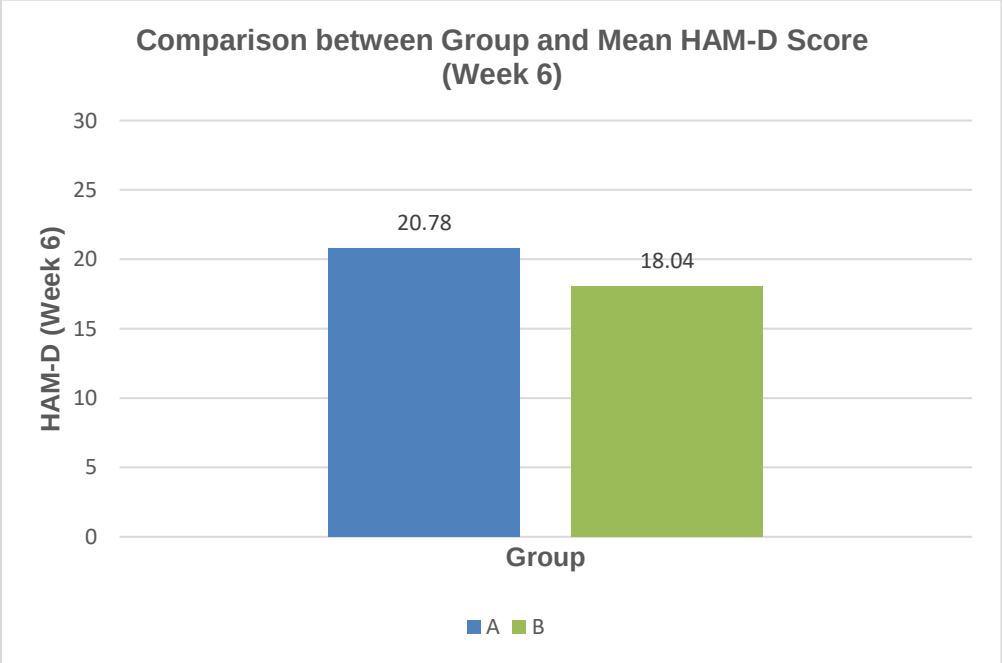

Figure 14 shows that mean HAM-D score at Week 6 is 20.78 in Group A and 18.04 in Group B and the change was significant (p<0.001).

Table 17: Comparison of the two Groups in Terms of change in HAM-D over time (n=100)

HAM-D	Group		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	A	B	
	Mean (SD)	Mean (SD)	
Week 0	28.00 (4.83)	28.04 (4.03)	0.945
Week 3	23.50 (6.22)	21.60 (3.84)	0.002
Week 6	20.78 (8.12)	18.04 (3.55)	<0.001
P Value for change in HAM-D over time within each group (Friedman Test)	<0.001	<0.001	
Overall P Value for comparison of change in HAM-D over time between the two groups (Generalized Estimating Equations)	0.004		

Table 17 shows Comparison of the two Groups in Terms of change in HAM-D over time and there was significant decrease in mean HAM-D score in group B as compared to group A at Week 3 (p=0.002) and at Week 6 (p<0.001).

Figure 15: Comparison (line diagram) of the two Groups in Terms of change in HAM-D over time (n=100)

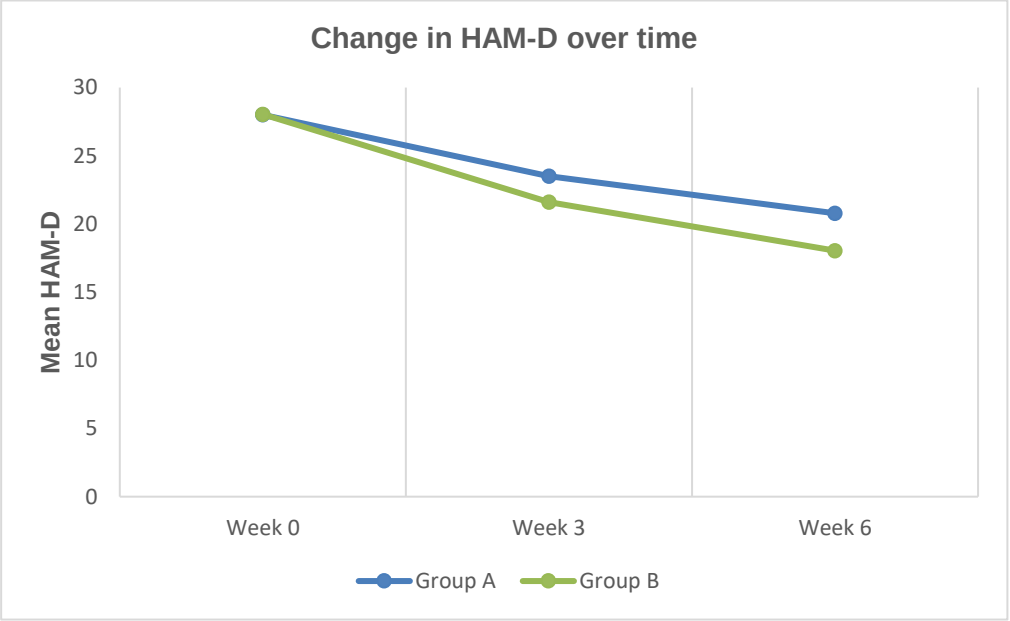

Figure 15 shows Comparison of the two Groups in Terms of change in HAM-D over time and there was significant decrease in mean HAM-D score in group B as compared to group A at Week 3 ($p=0.002$) and at Week 6 ($p<0.001$).

Table 18: Comparison of mean, median and range of MADRS scores at week 0, week 3 and week 6 (n=100)

	Week 0	Week 3	Week 6
Mean (SD)	41.45 (5.91)	35.69 (7.20)	29.32 (8.04)
Median (IQR)	42 (37-46)	35.5 (31.75-40.25)	30 (26-35)
Range	26 - 51	14 - 47	7 - 42

Table 18 shows that mean MADRS scores decreased from 41.45 at week 0 to 29.32 at week 6.

Table 19: Comparison of Group A & B in Terms of MADRS (Week 0) (n=100)

MADRS (Week 0)	Group		Wilcoxon-Mann-Whitney U Test	
	A	B	W	p value
Mean (SD)	41.56 (7.05)	41.34 (4.56)	1382.000	0.363
Median (IQR)	43.5 (38.25-48)	41 (37-45)		
Range	26 - 51	34 - 51		

Table 19 shows that mean MADRS score at Baseline (Week 0) is 41.56 in Group A and 41.34 in Group B and the change was insignificant (p=0.363).

Figure 16: Comparison of Group A & B in Terms of MADRS (Week 0) (n=100)

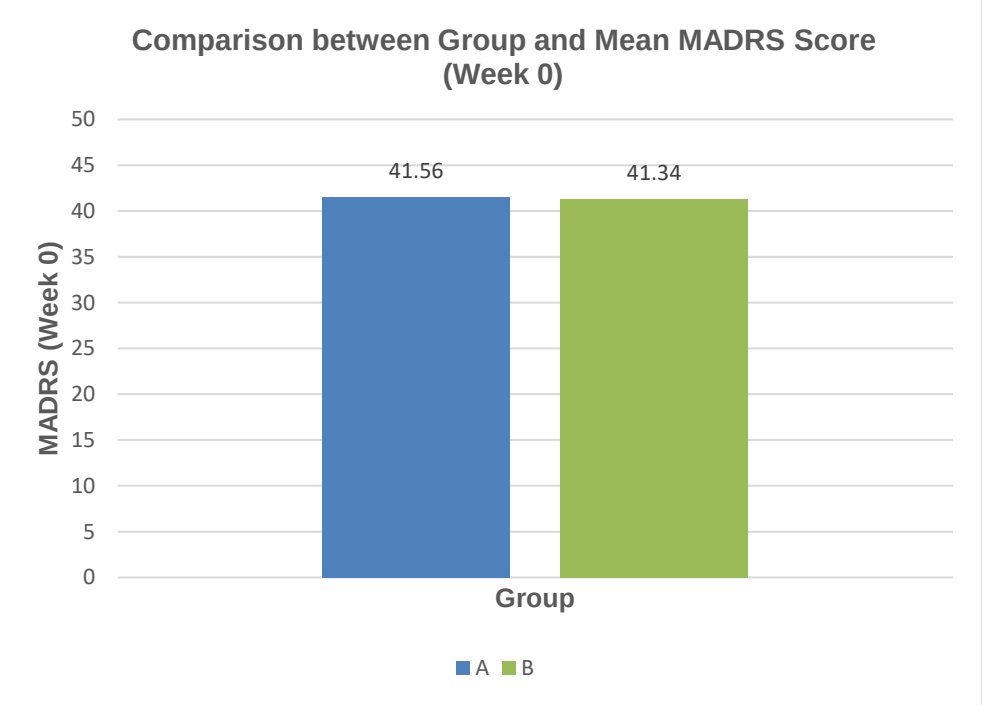

Figure 16 shows that mean MADRS score at Baseline (Week 0) is 41.56 in Group A and 41.34 in Group B and the change was insignificant (p=0.363).

Table 20: Comparison of Group A & B in Terms of MADRS (Week 3) (n=100)

MADRS (Week 3)	Group		Wilcoxon-Mann-Whitney U Test	
	A	B	W	p value
Mean (SD)	37.02 (9.16)	34.36 (4.16)	1677.500	0.003
Median (IQR)	40 (32.25-45.75)	34 (31.25-36.75)		
Range	14 - 47	26 - 46		

Table 20 shows that mean MADRS score at Week 3 is 37.02 in Group A and 34.36 in Group B and the change was significant (p=0.003).

Figure 17: Comparison of Group A & B in Terms of MADRS (Week 3) (n=100)

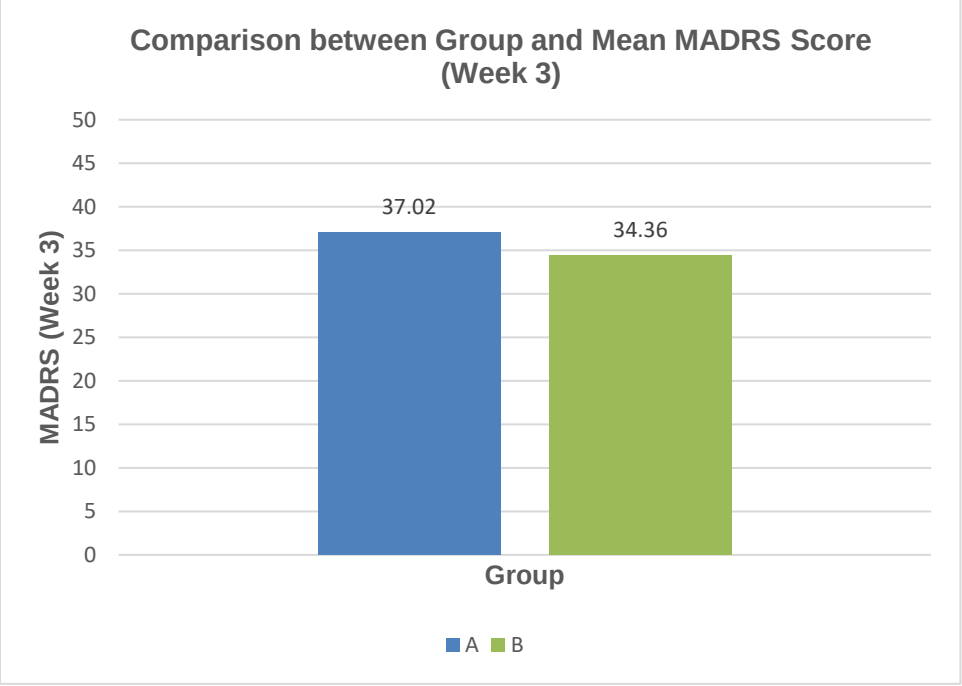

Figure 17 shows that mean MADRS score at Week 3 is 37.02 in Group A and 34.36 in Group B and the change was significant (p=0.003).

Table 21: Comparison of Group A & B in Terms of MADRS (Week 6) (n=97)

MADRS (Week 6)	Group		Wilcoxon-Mann-Whitney U Test	
	A	B	W	p value
Mean (SD)	30.22 (10.60)	28.40 (3.93)	1617.000	0.001
Median (IQR)	34 (29-38)	28.5 (26-30)		
Range	7 - 42	20 - 39		

Table 21 shows that mean MADRS score at Week 3 is 30.22 in Group A and 28.40 in Group B and the change was significant (p=0.001).

Figure 18: Comparison of Group A & B in Terms of MADRS (Week 6) (n=97)

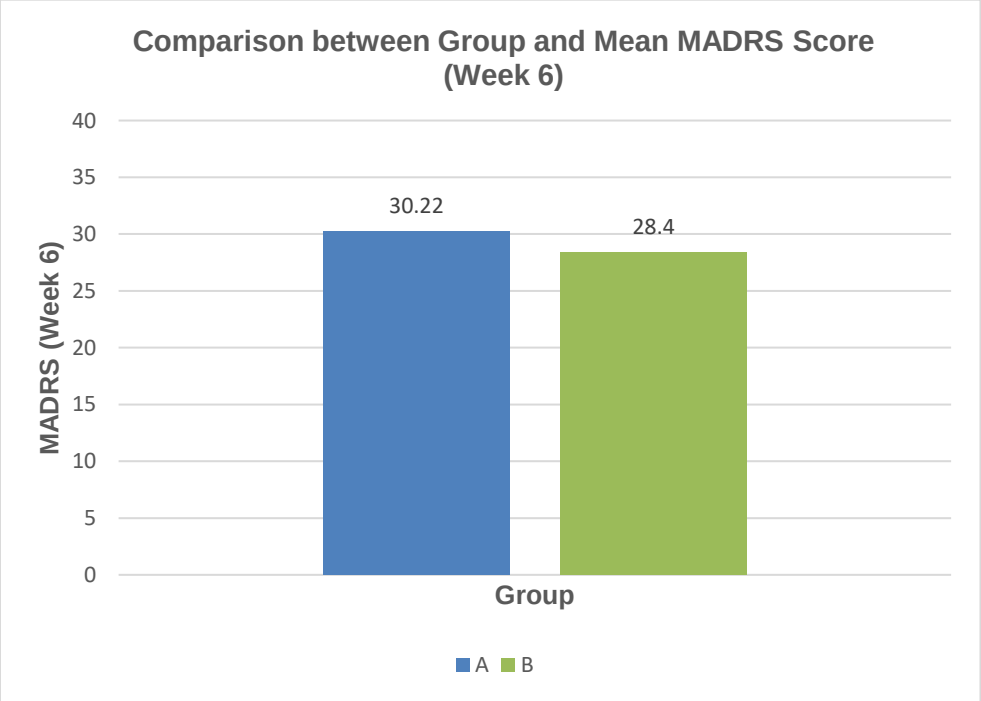

Figure 18 shows that mean MADRS score at Week 3 is 30.22 in Group A and 28.40 in Group B and the change was significant (p=0.001).

Table 22: Comparison of the two Groups in Terms of change in MADRS over time (n=100)

MADRS	Group		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	A	B	
	Mean (SD)	Mean (SD)	
Week 0	41.56 (7.05)	41.34 (4.56)	0.363
Week 3	37.02 (9.16)	34.36 (4.16)	0.003
Week 6	30.22 (10.60)	28.40 (3.93)	0.001
P Value for change in MADRS over time within each group (Friedman Test)	<0.001	<0.001	
Overall P Value for comparison of change in MADRS over time between the two groups (Generalized Estimating Equations)	<0.001		

Table 22 shows Comparison of the two Groups in Terms of change in MADRS over time and there was significant decrease in mean MADRS score in group B as compared to group A at Week 3 (p=0.003) and at Week 6 (p=0.001).

Figure 19: Comparison (line diagram) of the two Groups in Terms of change in MADRS over time (n=100)

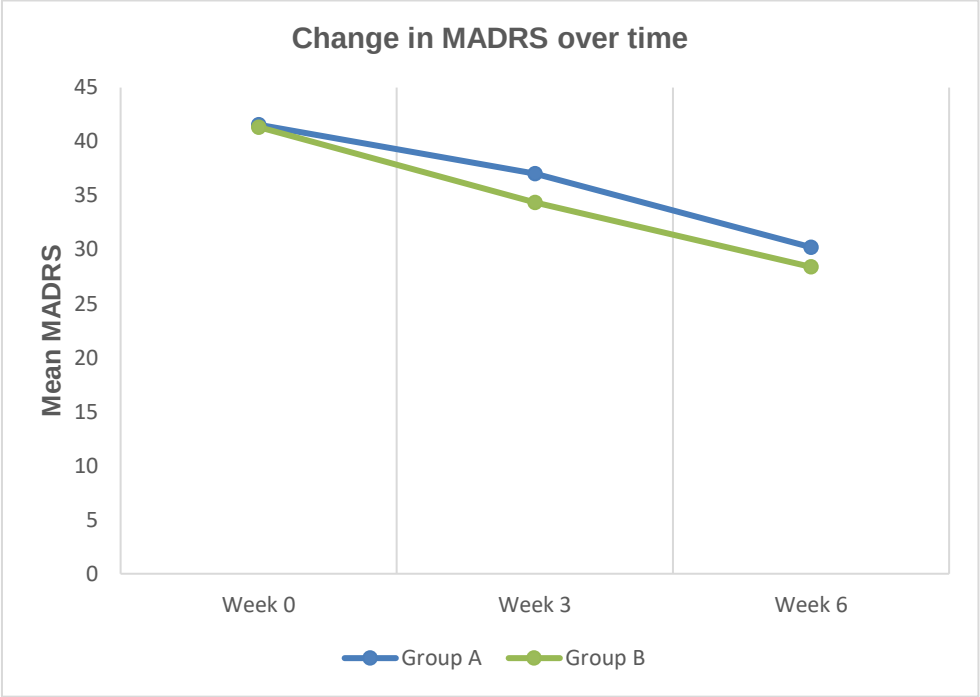

Figure 19 shows Comparison of the two Groups in Terms of change in MADRS over time and there was significant decrease in mean MADRS score in group B as compared to group A at Week 3 (p=0.003) and at Week 6 (p=0.001)

RESULTS

The study was a double blind randomized controlled trial, conducted on 100 patients of Major Depressive Disorder presenting to DMC&H who were already on treatment with antidepressants. Patients were allotted to consecutively receive either probiotic or the placebo for 6 weeks where both the patient and researcher were blinded. Unblinding was done after the study was completed which revealed that Group A is Placebo and Group B is the Probiotic capsule containing probiotic not less than 2.5 billion cells of *Lactobacillus acidophilus* (R0052), *Lactobacillus rhanmosus (R0011), Bifidobacterium Longum (R00175), Bacillus coagulans (SNZ1969)* and *Saccharomyces boulardii*. The data, thus, collected was tabulated and after analyzing, the following results were obtained.

TABLE 1 shows group wise distribution of patients. 50 patients were in Group: A and 50 patients were in Group: B.

TABLE 2 shows age wise distribution of the patients. Majority of patients (45%) were in age group 26-40 years, followed by 36% in 41-55 years age group, followed by 12% in 56-65 years age group and only 7% in 18-25 years age group.

It also shows association between group and age. Fisher's exact test was used to explore the association between 'Group' and 'Age' as more than 20% of the total number of cells had an expected count of less than 5.

There was no significant difference between the two groups in terms of distribution of Age ($\chi 2 = 4.086$, $p = 0.264$).

FIGURE 1 shows distribution of age between two Groups. It shows that majority of the patients in Group A (42%) were in 41-55 years age group, followed by 36% in 26-40 years age group, followed by 12% in 56-65 years age group and only 10% in 18-25 years age group and majority of the patients in Group B (54%) were in 26-40 years age

group, followed by 30% in 41-55 years age group, followed by 12% in 56-65 years age group and only 4% in 18-25 years age group in Group B (54%) were in 26-40 years age group.

TABLE 3 shows gender wise distribution of the patients. 56 patients were male and 44 were females.

It also shows association between group and gender. Chi-squared test was used to explore the association between 'Group' and 'Gender'.

There was no significant difference between the two groups in terms of distribution of Gender ($\chi 2 = 0.000$, $p = 1.000$).

Figure 2 shows distribution of gender between two groups. It shows that majority of the patients in both Group A and B (56%) were males and 44% were females in each group.

TABLE 4 shows education wise distribution of the patients. 23% patients were illiterate and rest 77% patients were literate 23% of the patients had studied till Primary School, 12% of the patients had studied till Middle School, 17% of the patients had studied till High School, 14% of the patients had studied till Higher Secondary/Diploma and another 6% of the patients were Graduates and rest 5% of the patients were Post-Graduates or had an honors degree.
It also shows association between group and education. Fisher's exact test was used to explore the association between 'Group' and 'Education' as more than 20% of the total number of cells had an expected count of less than 5.

There was no significant difference between the two groups in terms of distribution of Education ($\chi 2=4.327$, $p=0.660$).

Figure 3 shows distribution of education status between two groups. It shows that majority of the patients in Group A (26%) had studied upto primary school, followed by 20% who were illiterate, followed by 18% who were studied upto high school, followed by 12% who were studied

upto higher secondary, followed by 10% who were studied upto middle school and graduation, followed by 4% who were studied upto post graduate. Majority of the patients in Group B (26%) were illiterate, followed by 20% who were studied upto primary school, followed by 16% who were studied upto high school and higher secondary, followed by 14% who were studied upto middle school, followed by 6% who were studied upto post graduation and 2% who were studied upto graduation.

TABLE 5 shows occupation wise distribution of the patients. Majority of patients (64%) were employed- 27% of the patients were farmer, 7% of the patients were skilled workers, 16% of the patients were semi-skilled workers, 14% of the patients were businessman or self-employed, and 3% were unemployed, 5% were students, 27% homemakers and 1% were retired persons.

It also shows association between group and occupation. Fisher's exact test was used to explore the association between 'Group' and 'Occupation' as more than 20% of the total number of cells had an expected count of less than 5.

There was no significant difference between the two groups in terms of distribution of Occupation ($\chi 2=3.439$, $p=0.885$).

FIGURE 4 shows distribution of occupation between two groups. It shows that majority of the patients in Group A (30%) were farmers, followed by 26% were homemakers, followed by 18% were semi skilled workers, followed by 10% were businessman, followed by 8% were skilled workers, followed by 6% were students and 2% were unemployed. Majority of the patients in Group B (28%) were homemaker, followed by 24% were farmers, followed by 18% were businessman, followed by 14% were semi skilled workers, followed by 6% were skilled workers, followed by 4% were student and 4% were unemployed and 1% were retired.

TABLE 6 shows distribution of the patients in terms of monthly income

(Rs). Majority of the patients (44%) had low monthly income of less than Rs. 10,000, 26% had Monthly Income (Rs): 10,000-20,000, 15% had Monthly Income (Rs): 20,000-30,000, 7% had Monthly Income (Rs): 30,000-50,000 and 8% of the patients had Monthly Income (Rs): >50,000.

It also shows association between group and monthly income (Rs). Fisher's exact test was used to explore the association between 'Group' and 'Monthly Income (Rs)' as more than 20% of the total number of cells had an expected count of less than 5.

There was no significant difference between the two groups in terms of distribution of Monthly Income (Rs) ($\chi 2=1.506$, $p=0.840$).

FIGURE 5 shows distribution of monthly income (Rs) between two groups. It shows that majority of the patients in Group A (44%) had monthly income of less than Rs.10,000, followed by 28% having income 10,000-20,000, followed by 16% having income 20,000-30,000, followed by 8% having income >50,000, followed by 4% having income 30,000-50,000. Majority of the patients in Group B (44%) had monthly income of less than Rs.10,000, followed by 24% having income 10,000-20,000, followed by 14% having income 20,000-30,000, followed by 10% having income 30,000-50,000, followed by 8% having income >50,000.

TABLE 7 shows distribution of the patients in terms of family type. Maximum patients (58%) were living in a joint family, followed by 29% of patients living in extended family and only 13% were living in a nuclear family.

It also shows association between group and family type. Chi-squared test was used to explore the association between 'Group' and 'Family Type'.

There was no significant difference between the two groups in terms of distribution of Family Type ($\chi 2=0.180$, $p=0.914$).

FIGURE 6 shows distribution of family type between two groups. It shows that majority of the patients in Group A (56%) belonged to joint family, followed by 30% living in extended family, followed by only 14% living in nuclear family. Majority of the patients in Group B (60%) belonged to joint family, followed by 28% living in extended family, followed by only 12% living in nuclear family

TABLE 8 shows distribution of the patients in terms of marital status. Maximum patients (65%) were married, 30% were unmarried, 4% were divorced and only 1% were remarried.

It also shows association between group and marital status. Fisher's exact test was used to explore the association between 'Group' and 'Marital Status' as more than 20% of the total number of cells had an expected count of less than 5.

There was no significant difference between the two groups in terms of distribution of Marital Status ($\chi 2=2.149$, $p=0.635$).

FIGURE 7 shows distribution of marital status between two groups. It shows that majority of the patients in Group A (66%) were married, followed by 32% who were unmarried and 2% who were divorced. Majority of the patients in Group B (64%) were married, followed by 28% who were unmarried, 6% who were divorced and 1% were remarried.

TABLE 9 shows distribution of the patients in terms of family h/o psychiatric illness. Only 48% patients had family H/O psychiatric illness and 52% of the patients had no family H/O psychiatric illness.
It also shows association between group and family h/o psychiatric illness. Chi-squared test was used to explore the association between 'Group' and 'Family H/O Psychiatric Illness'.

There was no significant difference between the two groups in terms of distribution of Family H/O Psychiatric Illness ($\chi 2=0.160$, $p=0.689$).

FIGURE 8 shows distribution of family h/o psychiatric illness between two groups. It shows that in Group A, 50% patients had Family H/O Psychiatric Illness and 50% had no Family H/O any Psychiatric Illness. In Group B, 46% patients had Family H/O Psychiatric Illness and 54% had no Family H/O any Psychiatric Illness.

TABLE 10 shows distribution of the patients in terms of h/o self-harm or suicidal ideation. Majority of the patients (67%) had H/O self-harm or suicidal ideation and 33% of the patients had no H/O self-Harm or suicidal ideation.

It also shows association between group and h/o self-harm ideation. Chi-squared test was used to explore the association between 'Group' and 'H/O Self-Harm Ideation'.

There was no significant difference between the two groups in terms of distribution of H/O Self-Harm Ideation ($\chi 2=0.045$, $p=0.832$).

FIGURE 9 shows distribution of family h/o psychiatric illness between two groups. It shows that majority of the patients in Group A (66%) had H/O Self-Harm or Suicidal Ideation and 34% had no H/O Self-Harm or Suicidal Ideation. Majority of the patients in Group B (68%) had H/O Self-Harm or Suicidal Ideation and 32% had no H/O Self-Harm or Suicidal Ideation

TABLE 11 shows distribution of the patients in terms of compliance to treatment (week 6). For the purpose of this research, adequate compliance was defined as at least 85% treatment adherence to the regimen prescribed. 97 patients were compliant to treatment and 3 patients were non compliant to treatment at the end of 6 weeks.

It also shows association between group and compliance to treatment. Fisher's exact test was used to explore the association between 'Group' and 'Compliance to Drug/Placebo (Week 6)' as more than 20% of the total number of cells had an expected count of less than 5.

There was no significant difference between the two groups in terms of distribution of Compliance to Drug/Placebo (Week 6) ($\chi 2$=0.344, p=1.000).

FIGURE 10 shows distribution of the patients in terms of compliance to treatment (week 6) between two groups. It shows that in Group A, 98% patients were compliant to treatment and 2% were non compliant to treatment. In Group B, 96% patients were compliant to treatment and 4% patients were non compliant to treatment at the end of 6 weeks.

TABLE 12 shows distribution of the participants in terms of class of antidepressant. Majority of patients (94%) were prescribed SSRI antidepressant and only 6% were prescribed SNRI antidepressant.

It also shows association between group and class of antidepressant. Fisher's exact test was used to explore the association between 'Group' and 'Class of Antidepressant' as more than 20% of the total number of cells had an expected count of less than 5.

There was no significant difference between the two groups in terms of distribution of Class of Antidepressant ($\chi 2$=0.000, p=1.000).

FIGURE 11 shows distribution of the participants in terms of class of antidepressant between two groups. It shows that majority of patients (94%) in both groups each, were prescribed SSRI antidepressant and only 6% were prescribed SNRI antidepressant.

TABLE 13 shows comparison of mean, median and range of HAM-D scores at week 0, week 3 and week 6 (n=100).

The mean (SD) of HAM-D at week 0 was 28.02 (4.42), which decreased to 22.55 (5.23) at week 3 and further decreased to 19.42 (6.40) at end of week 6. The median (IQR) of HAM-D at week 0 was 29.00 (26-30.25), followed by 9.00 (17-24) at week 3 and 19.00 (17-24) at week 6. The HAM-D at week 0 ranged from 16-38, at week 3 ranged from 9-32 and at week 6 ranged from 4-31.

TABLE 14 and FIGURE 12 shows comparison of Group A & B in Terms of HAM-D (Week 0). The variable HAM-D (Week 0) was not normally distributed in the two subgroups of the variable Group. Thus, non-parametric tests (Wilcoxon-Mann-Whitney U Test) were used to make group comparisons. It shows that mean HAM-D score at baseline (Week 0) is 28.00 in Group A and 28.04 in Group B and there was no significant difference between the two groups in terms of HAM-D (Week 0) (W=1260.500, p=0.945).

TABLE 15 and FIGURE 13 shows comparison of Group A & B in Terms of HAM-D (Week 3). The variable HAM-D (Week 3) was not normally distributed in the two subgroups of the variable Group. Thus, non-parametric tests (Wilcoxon-Mann-Whitney U Test) were used to make group comparisons. It shows that mean HAM-D score at Week 3 is 23.50 in Group A and 21.60 in Group B and there was a significant difference between the two groups in terms of HAM-D (Week 3) (W=1699.000, p=0.002).

TABLE 16 and FIGURE 14 shows comparison of Group A & B in Terms of HAM-D (Week 6). The variable HAM-D (Week 6) was not normally distributed in the two subgroups of the variable Group. Thus, non-parametric tests (Wilcoxon-Mann-Whitney U Test) were used to make group comparisons. It shows that mean HAM-D score at Week 6 is 20.78 in Group A and 18.04 in Group B and there was a significant difference between the two groups in terms of HAM-D (Week 6) (W=1741.000, p=<0.001).

TABLE 17 and FIGURE 15 (line diagram) shows comparison of the two Groups in Terms of change in HAM-D over time.

Non-Parametric tests were used to make statistical inference as data were not normally distributed. Wilcoxon-Mann-Whitney Test was used to compare the two groups in terms of HAM-D at each of the timepoints (right-most column in the table above). Friedman test was used to explore the change in HAM-D over time within each group (second-last

row in the table above). Generalized Estimating Equations method was used to explore the difference in change in HAM-D between the two groups over time (last row in the table above).

The two groups differed significantly in terms of HAM-D at the following timepoints: Week 3, Week 6.

In Group: A, the mean HAM-D decreased from a maximum of 28.00 at the Week 0 timepoint to a minimum of 20.78 at the Week 6 timepoint. This change was statistically significant (Friedman Test: $\chi 2=98.0$, $p<0.001$).

In Group: B, the mean HAM-D decreased from a maximum of 28.04 at the Week 0 timepoint to a minimum of 18.04 at the Week 6 timepoint. This change was statistically significant (Friedman Test: $\chi 2=96.0$, $p<0.001$).

The overall change in HAM-D over time was compared in the two groups using the Generalized Estimating Equations method. There was a significant difference in the trend of HAM-D over time between the two groups ($p=0.004$).

TABLE 18 shows comparison of mean, median and range of **MADRS** scores at week 0, week 3 and week 6 (n=100).

The mean (SD) of MADRS at week 0 was 41.45 (5.91), which decreased to 35.69 (7.20) at week 3 and further decreased to 29.32 (8.04) at end of week 6. The median (IQR) of MADRS at week 0 was 42.00 (37-46), followed by 35.50 (31.75-40.25) at week 3 and 30(26-35) at week 6. The MADRS at week 0 ranged from 26-51, at week 3 ranged from 14-47 and at week 6 ranged from 7-42.

TABLE 19 and FIGURE 16 shows comparison of Group A & B in Terms of MADRS (Week 0). The variable MADRS (Week 0) was not normally distributed in the two subgroups of the variable Group. Thus, non-parametric tests (Wilcoxon-Mann-Whitney U Test) were used to

make group comparisons. It shows that mean MADRS score at baseline (Week 0) is 41.56 in Group A and 41.34 in Group B and there was no significant difference between the twogroups in terms of MADRS (Week 0) (W=1382.000, p=0.363).

TABLE 20 and FIGURE 17 shows comparison of Group A & B in Terms of MADRS (Week 3). The variable MADRS (Week 3) was not normally distributed in the two subgroups of the variable Group. Thus, non-parametric tests (Wilcoxon-Mann-Whitney U Test) were used to make group comparisons. It shows that mean MADRS score at Week 3 is 37.02 in Group A and 34.36 in Group B and there was a significant difference between the two groups in terms of MADRS (Week 3) (W=1677.500, p=0.003).

TABLE 21 and FIGURE 18 shows comparison of Group A & B in Terms of MADRS (Week 6). The variable MADRS (Week 6) was not normally distributed in the two subgroups of the variable Group. Thus, non-parametric tests (Wilcoxon-Mann-Whitney U Test) were used to make group comparisons. It shows that mean MADRS score at Week 6 is 30.22 in Group A and 28.40 in Group B and there was a significant difference between the two groups in terms of MADRS (Week 6) (W=1617.000, p=0.001).

TABLE 22 and FIGURE 19 (line diagram) shows comparison of the two Groups in Terms of change in MADRS over time.

Non-Parametric tests were used to make statistical inference as data were not normally distributed. Wilcoxon-Mann-Whitney Test was used to compare the two groups in terms of MADRS at each of the timepoints (right-most column in the table above). Friedman test was used to explore the change in MADRS over time within each group (second-last row in the table above). Generalized Estimating Equations method was used to explore the difference in change in MADRS between the two groups over time (last row in the table above).

The two groups differed significantly in terms of MADRS at the

following timepoints: Week 3, Week 6.

In Group: A, the mean MADRS decreased from a maximum of 41.56 at the Week 0 timepoint to a minimum of 30.22 at the Week 6 timepoint. This change was statistically significant (Friedman Test: $\chi 2=98.0$, $p<0.001$).

In Group: B, the mean MADRS decreased from a maximum of 41.34 at the Week 0 timepoint to a minimum of 28.40 at the Week 6 timepoint. This change was statistically significant (Friedman Test: $\chi 2=95.5$, $p<0.001$).

The overall change in MADRS over time was compared in the two groups using the Generalized Estimating Equations method. There was a significant difference in the trend of MADRS over time between the two groups ($p<0.001$).

DISCUSSION

Major Depressive Disorder is a burdensome disorder which was earlier accepted as a disorder of reduced monoaminergic activity in the brain but it has now been established that disturbance in the equilibrium of the gut microbiota has also been involved in the pathophysiology of depression [13]. Inflammation, oxidative stress, dysregulated function of the hypothalamic-pituitary-adrenal axis, and decreased neurotrophins including brain-derived neurotrophic factor are the major pathophysiological pathways that play a role in pathogenesis of Major Depressive Disorder [6]. Inflammation can compromise normal barriers protecting the body from pathogenic gut bacteria, resulting in intestinal permeability also known as ‘leaky gut', and even leakage at the blood-brain barrier. One mechanism whereby this occurs is tumor necrosis factor alpha (TNFa) induction of shedding and apoptosis of intestinal epithelial cells. Upregulation of inflammatory cytokines has downstream consequences in brain, including shunting of tryptophan away from serotonin synthesis toward the kynurenine pathway as well as excitotoxic and neurotoxic effects [13]. Probiotics have the potential to modulate the gut microbiome [16]. The understanding of the gut-brain axis has evolved a new era of probiotics referred to as “psychobiotics” for their influence on the CNS, cognition, and behavior [3].

This study was conducted to find out the effect of probiotic supplementation on symptoms of depression in patients of Major Depressive Disorder who are already on antidepressants. Since depression is now being regarded as an inflammatory disease, it is necessary to develop ways to decrease it. This study is unique in the sense that it aims to find out a nutritional intervention that is based on the intake of probiotics that may successfully help in prevention and treatment of depressive disorders. This intervention is harmless, easily available, and an effective way to manage depressive symptoms.

This study was conducted on 100 patients of Major Depressive Disorder presenting to DMC & H who were already on treatment with

antidepressants. Patients were allotted to consecutively receive either probiotic or the placebo for 6 weeks where both the patient and researcher were blinded. Dietary intake of patients was not significantly different between the two groups. Patients were screened using MINI as having only MDD and severity of depression was assessed at 0, 3 & 6 weeks each using HAM-D and MADRS scales. Antidepressant treatment was continued and Probiotic and Placebo were administered as an add on treatment. Unblinding was done after the study was completed. On unblinding of trial at the end of study, it was revealed that group A is placebo group and group B is the probiotic group containing probitoic capsule Sporlac plus-containing probiotic not less than 2.5 billion cells of *Lactobacillus acidophilus* (R 0052), *Lactobacillus rhamnosus* (R 0011), *Bifidobacterium longum* (R 00175), *Bacillus coagulans* (SNZ 1969), and *Saccharomyces boulardii*.

In this study, majority of the patients in placebo group (42%) were in 41-55 years age group and in probiotic group (54%) were in 26-40 years age group. Both the groups were comparable and no biostatistically significant difference was observed among both the groups in terms of age. In a study conducted by Akkasheh et al [15], the age distribution of the participants was similar to our study.

In this study, in both groups, 56% of the participants were male and 44% of the participants were female. Both the groups were biostatistically similar and no significant difference was observed among both the groups in terms of gender distribution.

In this study, majority of the patients, 80% in placebo group and 74% in probiotic group were literate. Literacy rates in both the groups were similar and no statistically significant variation was seen among both the groups in terms of education status and thus, both the groups were comparable.

In this study, majority of the patients in placebo group (66%) and probiotic group (64%) were married. Marital status of both the groups

was similar and there was no statistically significant difference among both the groups, therefore, both the groups were comparable. The results were comparable to a study by done by Östlund-Lagerström et al [58].

In the current study, in placebo group- 50% patients had Family H/O Psychiatric Illness and in probiotic group, 48% patients had Family H/O any Psychiatric Illness. Both the groups had no biostatistically significant variation and hence, were comparable.

EFFECT ON HAMD SCORES

In our study, the patients that were in the placebo group had a mean HAM-D score of 28.00 at Week 0, while in probiotic group, mean HAM-D score at week 0 is 28.04. There was no biostatistically significant difference between the groups in terms of HAM-D at Week 0 and hence, both the groups were comparable (W=1260.500, p=0.945).

In our study, the patients in placebo group had mean HAM-D score of 23.50 at Week 3 which decreased from mean score of 28.00 at baseline. While, the patients in probiotic group had mean HAM-D score of 21.60 at Week 3 which decreased from mean score of 28.04 at baseline. In contrast to findings in placebo group, the group containing probiotics had shown a greater improvement in depressive symptoms as assessed by mean HAM-D scores. Our study results showed that there was a significant decrease in HAM-D score in probiotic group as compared to placebo group at week 3 (W=1699.000, p=0.002).

The present study showed that at the end of Week 6, the placebo group had mean HAM-D score of 20.78 which decreased from mean HAM-D score of 23.50 at week 3. Whereas, the probiotic group had mean HAM-D score of 18.04 at the end of week 6 which decreased from mean HAM-D score of 21.60 at week 3. As was the case in week 3, Week 6 continued the same trend where probiotic group showed greater improvement in depressive symptoms as compared to placebo group. The findings from the present study showed that there was a

significant decrease in HAM-D score in probiotic group as compared to placebo group at the end of Week 6 (W=1741.000, p<0.001).

Our results were in agreement to a study conducted by Ghorbani et al[26], results of which showed a greater reduction in HAM-D score in synbiotic treated patients (Mean+-SD = -19.25+-1.71) compared to placebo taking group (Mean+-SD = -17.75+-2.05; p=0.024). It was concluded that high plasma concentrations of proinflammatory cytokines detected in depressive patients were responsible for depressive symptoms. Firstly, given the role of HPA axis in regulating moods and behavior, synbiotic therapy can modulate its stress responses and improve depression symptoms. Secondly, the probiotic contents in synbiotic agents negatively affect the growth of pathogens and their components including lipopolysaccharide in the intestine which result in enhancement of the gut microbiota, which might alter the neurotransmitter levels in the Central nervous system especially GABA and serotonin.

EFFECT ON MADRS SCORE

In this present study, the patients of placebo group had mean MADRS score of 41.56 at Week 0 (baseline) and the mean MADRS score of probiotic group is 41.34. There was no significant difference between both the groups in terms of MADRS at Week 0 and hence, both the groups were comparable. (W=1382.000, p=0.363).

In our study, the placebo group had mean MADRS score of 37.02 at Week 3 which decreased from mean MADRS score of 41.56 at baseline. Whereas, the patients in probiotic group had mean MADRS score of 34.36 at Week 3 which decreased from mean MADRS score of 41.34 at baseline. The Group B had shown a greater improvement in depressive symptoms as compared to Group A as assessed by mean MADRS scores. The results of present study showed that there was a significant decrease in mean MADRS score at week 3 in probiotic group as compared to placebo group (W=1677.500,p=0.003).

The present study showed that at the end of Week 6, the placebo group had mean MADRS score of 30.22 at the end of Week 6 which decreased from mean MADRS score of 37.02 at week 3. Whereas, the probiotic group had mean MADRS score of 28.40 at Week 6 which decreased from mean MADRS score of 34.36 at week 3. Similar to week 3, Week 6 continued the same trend where probiotic group showed greater improvement in depressive symptoms as compared to placebo group. The findings from the present study showed that there was a significant decrease in MADRS score in probiotic group as compared to placebo group at the end of Week 6 (W=1617.000, p=0.001).

Additionally, In a study conducted by Jacka et al [44] it was determined that even dietary improvement may provide an efficacious and accessible treatment strategy for the management of MDD. It showed that the dietary support group demonstrated significantly greater improvement between baseline and 12 weeks on the MADRS scores than the social support control group. It was proposed that dietary improvement may influence depressive illness through its action on inflammatory and oxidative stress pathways, as well as brain plasticity. Each of these pathways is suggested to play a role in depression and is also influenced by diet quality.

Our study results are similar to a study conducted by Akkasheh et al [15] in which, after 8 weeks of intervention, patients who received probiotic supplements had significantly decreased Beck Depression Inventory total scores (p=0.001) compared with the placebo. It was concluded that the administration of probiotics might result in improved symptoms of depression through increased plasma tryptophan levels, decreased serotonin metabolite concentrations in the frontal cortex, and decreased dopamine metabolite concentrations in the amygdaloid cortex. Probiotic bacteria through fermenting dietary ingredients might lead to specific changes in the composition and activity of the gastrointestinal microbiota, which might result in improved peripheral

(gastrointestinal) and central (psychological) symptoms. Probiotics influence both the enteric nervous system and the central nervous system in addition to their effects on the mucosal immune system by modifying the gastrointestinal tract microbiome.

The results of our study are similar with a study conducted by Kazemi et al [16], in which a total of 81 subjects of depression completed the trial (28 in the probiotic group, 27 in the prebiotic group, and 26 in the placebo group). The study results showed that from baseline to 8 weeks, probiotic supplementation resulted in a significant decrease in Becks Depression Inventory score (17.39-9.1) compared to the placebo (18.18-15.55) and prebiotic (19.72-14.14) supplementation (p=0.042).

Our study results are comparable to a study conducted by Steenbergen et al [17] which showed that as compared to participants who received the placebo intervention, participants who received the 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and aggressive thoughts. It has been proposed that intestinal microbiota increase plasma tryptophan levels, and hereby potentially facilitate serotonin turnover in the brain. It has been also proposed that an increased intestinal permeability can induce depressive symptoms, possibly by endotoxin activated inflammatory pathways or via direct activation of glial and neural cells that carry Toll-like receptors and are hereby responsive to a wide range of microbial products. Given that certain probiotics have been found to improve the epithelial barrier function and hereby decrease permeability, this mechanism might account for the beneficial effects of probiotics on cognitive reactivity.

Our study results are in line with a study conducted by Miyaoka et al [25] the results showed CBM588 (Clostridium butyricum MIYAIRI 588) in combination with antidepressants (fluvoxamine, paroxetine, escitalopram, duloxetine, and sertraline) provided significant

improvement in treatment resistant depression. Impaired neuroprotection, decreased neuronal survival and disordered neurogenesis in the hippocampus are involved in pathogenesis of major depression and are now considered to be therapeutic targets for major depression. CBM588 may exert potential antidepressant effects through its robust neuroprotective activities, which include neurogenesis, antioxidation, anti glutamate excitotoxicity, and direct regulation of proinflammatory agents.

Furthermore, In a study by Mohammadi et al [38] it was demonstrated that the mode of probiotic administered might also play a role in the way these agents act. After 6 weeks of intervention, a significant improvement of GHQ (general health questionnaire) was observed in the probiotic yogurt (p=0.007) and in the probiotic capsule group (p=0.001), as well as a significant improvement in DASS (depression anxiety and stress scale) scores in the probiotic yogurt (p=0.02) and the probiotic capsule group (p=0.006). However, there was no significant improvement in the conventional yogurt group (p=0.05 for GHQ and p=0.08 for DASS) which is in concordance with our study. It was proposed that there was influence of several factors that must be considered on activity and viability of probiotics in the consumer's gastrointestinal (GI) tract including the physiologic state of the probiotic added; the physicochemical conditions of product storage (e.g., temperature, acidity, available carbohydrate content, nitrogen sources, mineral content, water activity, and oxygen content) and possible interactions of the probiotics with the starter cultures (e.g., bacteriocin production, antagonism, and synergism).

Furthermore, it was proposed that probiotics may have beneficial effect on anxiety symptoms as well. In a study conducted by Hilimire et al [54] Fermented food consumption was negatively correlated with social anxiety indicating that those who consume more fermented foods containing probiotic bacteria have lower levels of social anxiety although depression wasn't assessed in these participants. In a study

conducted by Rao et al [55], there was a significant decrease in anxiety symptoms among those taking the probiotic vs controls (p=0.01). It was hypothesized that the fermented foods assessed in the current study reduce anxiety because they contain probiotics.

In a study conducted by Kouchaki et al [62] their findings further reinforced the results of our study. Compared with the placebo, probiotic intake improved beck depression inventory ($p<0.001$), general health 35 questionnaire ($p<0.001$) and depression anxiety and stress scale (p=0.001). Their study proposed that probiotics intake result in improved disability, symptoms of mental health and metabolic indicators by their effect on neuronal circuits and through the effect on gene expression.

In a study conducted by Marotta et al [63] 38 healthy volunteers assigned to an experimental or control group consumed a daily dose of a probiotic mixture (containing Lactobacillus fermentum LF16, L. rhamnosus LR06, L. plantarum LP01, and Bifidobacterium longum BL04) or placebo, respectively, for 6 weeks. The results were in line with our study as it showed a significant improvement in mood was observed in the experimental group, with a reduction in depressive mood state, anger, and fatigue, and an improvement in sleep quality. It was proposed that probiotics, by interacting with intestinal microbiota, facilitated the production of precursors of neuroactive substances involved in modulating emotional processing, sleep, and other brain functions.

Our study results were similar with a study conducted by Chahwan et al [64]. 71 participants were randomly allocated to either probiotic or placebo, which was, consumed daily over eight weeks. Participants in the probiotic group demonstrated a significantly greater reduction in cognitive reactivity compared with the placebo group which is a marker of depression.

In a study conducted by Slykerman et al [65] the results showed that the

participants in the probiotic treatment group Lactobacillus rhamnosus HN001 (HN001), reported significantly lower depression scores and anxiety scores than those in the placebo group, (p = 0·002). Changes in the expression of the neurotransmitter GABA receptors in brain have been demonstrated along with changes in anxiety-related behaviour with L. rhamnosus treatment.

In specific probiotic studies where individual strains were studied, a study conducted by Majeed et al [66] showed significant change (p=0.01) in favour of the B. coagulans MTCC 5856 for the primary efficacy measured on Hamilton Rating Scale for Depression (HAM-D), Montgomery-Asberg Depression Rating Scale (MADRS). It is suggested that the production of certain neurotransmitters, hormones, and immune- and neuropeptides and short-chain fatty acids by the probiotics may help in alleviating depression symptoms. Likewise, probiotic strain *B. coagulans* MTCC 5856 has also been reported to produce short-chain fatty acids (acetic, propionic and butyric acid), antimicrobial and anti-inflammatory substances which could be the possible mechanism of action in alleviating depression symptoms.

On the contrary, however, it should be noted that a variation was observed in a study conducted by Chung et al [51] where administration of Lactobacillus helveticus-fermented milk (LHFM) was done which showed that there were no significant effects of LHFM consumption on perceived stress scale (PSS) & geriatric depression scale-short form (GDS-SF). Although, it should be noted that they studied fermented milk instead of probiotic supplements used in present study.

In a study conducted by Seigel et al [56], a variation was found as it showed that 7-days consumption of B. longum does not reduce stress, depressive symptoms, or anxiety in generally healthy young adults. However, it could be because of an inadequate follow up time period of 7 days. The acute time period utilized in the present study was not long enough to allow for the bacteria introduced to proliferate enough to observe a measurable effect. As shown from results from the present

study, it takes time for improvement in depressive scores as scores started decreasing from week 3 and week 6 onwards.

Our study results were inconsistent with a study conducted by Romijn et al [57], as no significant difference was found between the probiotic and placebo groups on MADRS scale. At end-point, 9 of those in the probiotic group showed a ⩾60% change on the Montgomery–Åsberg Depression Rating Scale, compared to 10 of those in the placebo group. It is possible that the lack of observed effect of probiotics on psychological outcomes was due to the size of the sample (n=79 with 10 dropouts), and the severity as well as chronicity of the sample as the sample reported continuous low mood lasting for more than 2 years.

In contrast to the present study, a difference was observed in a study conducted by Östlund-Lagerström et al [58]. No persistent significant effects were observed on anxiety and stress after 12 weeks of intervention of either probiotic or placebo product. It should, however, be noted that the results were based on self-reported data and thus, rely on the respondents' accurate understanding and interpretation on the questions asked.

In the present study, we examined the beneficial effects of probiotic administration on HAM-D and MADRS scores in patients with MDD. The main findings were that probiotic supplementation improved the HAM-D and MADRS scores as compared to placebo group. The current study showed that the effect of the probiotics was seen as early as third week of the treatment regimen. The probable reasons of the outcome measures seen in probiotic group are complex and multifactorial. The administration of probiotics might result in improved symptoms of depression through the following mechanisms-

Bacterial metabolism of carbohydrates and proteins

Dietary carbohydrates, including indigestible oligosaccharides, are a common substrate for gut bacteria, which transform them into short

chain fatty acids (SCFAs), including acetate, propionate, butyrate and valerate. These substances serve as energy sources for the host and for other bacterial species. SCFAs also trigger differentiation of T cells and can function as histone deacetylase inhibitors, which as a class have immunosuppressive and anti-inflammatory functions and have been proposed as potential novel antidepressants.

Gut dysbiosis

Dysbiosis that results in increased putrefaction has been implicated in the pathogenesis of depression. We may speculate that this imbalance of gut microbiota can result in a relatively lower capacity to metabolize carbohydrates and higher capacity for protein metabolism may have a role in the pathogenesis of MDD. Lower SCFA could contribute to depressive symptomatology as a result of lower energy and altered neurotransmission.

It is also plausible that depression may cause digestive problems and dysbiosis either through altered dietary choices or other mechanisms. One hypothesis could be that in some individuals, depression confers a decreased ability to digest proteins; the resulting increased residual protein in the colon would give an advantage to higher concentrations of microbes which prefer proteins as substrates, leading to higher putrefaction and inflammation [13]. Probiotics help stabilize the imbalance of gut microbiota and hence, may exert antidepressant effects.

Biosynthesis of micronutrients

Water-soluble vitamins from the diet may be absorbed from the small intestine, including ascorbate (vitamin C), biotin (B7), folate (B9), niacin (B3), pantothenic acid (B5) pyridoxine (B6), riboflavin (B2) and thiamine (B1). However, a number of these water-soluble vitamins also can be generated by microbiota and absorbed in the colon and thereby affect the host. Therefore, dysbiosis resulting in low production of micronutrients could contribute to depression pathophysiology, particularly in the case of depressed patients with poor nutritional

intake [13].

Regulation of inflammation

Inflammation can compromise normal barriers protecting the body from pathogenic gut bacteria, resulting in increased intestinal permeability, or 'leaky gut', and even leakage at the blood-brain barrier. One mechanism whereby this occurs is tumor necrosis factor alpha (TNFa) induction of shedding and apopotosis of intestinal epithelial cells. This has been suggested as an explanation for the high association of inflammatory gastrointestinal disorders with depression. Gut dysbiosis promotion of inflammation may contribute to multiple pathways in the central nervous system that are implicated in the development of depression. Upregulation of inflammatory cytokines has downstream consequences in brain, including shunting of tryptophan away from serotonin synthesis toward the kynurenine pathway as well as excitotoxic and neurotoxic effects [13].

Probiotics hinder lipopolysaccharide producing pathogenic commensal bacteria, and reduce the subsequent lipopolysaccharide induced endotoxemia and inflammation. Also, probiotics improve intercellular integrity of tight junctions, leading to decreased para-cellular permeability and its consequent endotoxemia or inflammation. Inflammation reduces BDNF gene expression. BDNF is a neurotrophin which plays a critical role in neuroplasticity, and has been suggested as a biomarker for depression and treatment response [6]. Given that certain probiotics have been found to improve the epithelial barrier function and hereby decrease permeability, this mechanism might account for the beneficial effects of probiotics on depressive symptoms[17].

Suppression of Hypothalamic-Pituitary-Adrenal Axis

Depression is a stress-related mood disorder associated with a disrupted HPA axis. Stress results in activation of the hypothalamic-pituitary-adrenal axis by bringing about the release of corticotropin releasing hormone (CRH) and vasopressin (AVP) together with altered

gut barrier function. Probiotics act by suppressing the hypothalamic-pituitary-adrenal axis activity and exert the antidepressant action [21].

Neurotransmitter synthesis

Gut microbiota have been found to make precursors to neurotransmitters, such as tryptamine, and neurotransmitters including GABA, serotonin, norepinephrine, and dopamine. Several bacterial strains are known to produce serotonin directly in the hippocampus, along with increased peripheral levels of tryptophan, a serotonin precursor, suggesting the possibility of a peripheral origin to this effect. Modulation of neurotransmitter production is one possible means by which the gut microbiome may affect the brain, with relevance to depression [13].

In its metabolic pathway, tryptophan can either be converted to serotonin (a neurotransmitter with anti-depressant activities), or produce Kynurenine which is a substance with the potential to disrupt neurotransmitter balance, and might cause neuro-inflammation, impair the brain function, and contribute to development of depression. In addition, probiotic bacteria are capable of producing tryptophan, GABA and serotonin from their precursors [6].

To conclude, Major depressive disorder is now considered a neuroimmunological disorder that comprises not only the traditional monoamine deficiency, but also a persistent low grade inflammation, oxidative stress, dysregulated function of the hypothalamic-pituitary-adrenal (HPA) axis, and decreased neurotrophins including brain-derived neurotrophic factor (BDNF). These are now being regarded as major pathophysiological pathways that play a role in pathogenesis of MDD. Essentially, what we eat influences the composition of our gut microbiota and thus regulates the metabolites produced. When eating a healthy diet rich in probiotics, we feed the beneficial microbes in our gut and they return the favor by producing anti-inflammatory metabolites, energy, and beneficial neurochemicals [3]. Individuals with major depressive disorder have raised levels of a variety of

inflammatory markers including interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor - alpha (TNF-a), and C-reactive protein (CRP). The gut microbiota is now being viewed as a virtual endocrine organ that has bidirectional communication with the central nervous system through the microbiome-gut-brain axis [67]. The main mechanisms by which probiotics may improve depressive symptoms include modulation of neurotransmitters and inflammation [16]. Probiotics could directly, or through influence of other gut microbes, play a role in depression by inhibiting gut and systemicinflammation and thus, activating the microbiome-gut-brainaxis [67]. These metabolites reduce systemic inflammation by regulating the immune system and calming the HPA axis, which helps bring the body back into homeostasis and reduces depressive symptoms. Probiotics directly protect the intestinal barrier and also, limit bacterial overgrowth in the small intestine [67]. The beneficial neurochemicals produced by the microflora are able to pass the blood-brain barrier and regulate levels of BDNF, GABA, dopamine, and serotonin in the brain, thus reducing depressive symptoms [3].

LIMITATIONS

A limitation of the present study was that the sample size was limited to100 participants. Also, the sample was limited to one hospital setting. It should be noted that in this study, probiotics and antidepressants were prescribed together, the specific beneficial effects of each drug to the improvement in symptoms of depression cannot be evaluated. Although, prior to the initiation of treatment, it was advised to the patients that there should not be any changes in their dietary habits but the adherence to that was not strictly monitored, therefore, the possibility of subject's diet having impact on the results cannot be ruled out.

SUMMARY AND CONCLUSION

The present study was conducted to study the effect of probiotic supplementation on the symptoms of depression in patients of major depressive disorder already on antidepressants. In our study we noted the following points:-

- Maximum (45.0%) of the participants belonged to Age group- 26-40 Years.
- 56.0% of the participants were Male and 44.0% of the participants were Female.
- 23.0% of the participants were Illiterate and 77% were literate.
- 27.0% of the participants were Farmer and 27.0% of the participants were Homemaker.
- Maximum (44.0%) of the participants had Monthly Income (Rs): Upto 10,000 and 41.0% of the participants belonged to Upper Lower Socio-Economic status.
- 58.0% of the participants had Joint family.
- 65.0% of the participants were Married
- 48.0% of the participants had Family H/O Psychiatric Illness.
- 67.0% of the participants had H/O Self-Harm or Suicidal Ideation.
- 97.0% of the participants were Compliant to Antidepressants and drug/placebo at the end of study (Week 6).
- In terms of socio-demographic variables, both the groups were statistically similar with no significant variation.
- In Placebo Group, the mean±SD HAM-D score decreased from 28.00±4.83 at the Week 0 timepoint to 20.78±8.12 at the Week 6 time point whereas in Probiotic Group, the mean±SD HAM-D score decreased from 28.04±4.03 at the Week 0 time point to 18.04±3.55 at the Week 6 time point.
- Thus, Probiotic Group showed greater improvement in HAM-D scores as compared to Placebo Group. The results showed that there was a significant decrease in HAM-D score in probiotic group as compared to placebo group at the end of Week 6 ($W=1741.000$, $p<0.001$).

- In Placebo Group, the mean±SD MADRS score decreased from 41.56±7.05 at the Week 0 time point to 30.22±10.60 at the Week 6 time point whereas In Probiotic Group, the mean±SD MADRS score decreased from 41.34±4.56 at the Week 0 timepoint to 28.40±3.93 at the Week 6 time point.
- Therefore, Probiotic Group showed greater improvement in MADRS scores as compared to Placebo Group. The results of this study showed that there was a significant decrease in MADRS score in probiotic group as compared to placebo group at the end of Week 6 (W=1617.000, p=0.001).
- Based on the findings of this study, it is concluded that probiotic capsule used in this study, containing probiotic not less than 2.5 billion cells of Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Bacillus coagulans, and Saccharomyces boulardii, has a beneficial effect as an adjuvant treatment in patients of Major Depressive Disorder.

FUTURE DIRECTIONS

In the future, this topic must be explored further from a more functional pathway by implementation of large scale nutritional interventions along with probiotic supplementation to modify depression [3].It would be useful for such evidence to be further researched and established through future studies so that the clinicians can successfully consider probiotics as add-on treatment in MDD [7]. Therefore, further studies are required that recruit each of the strains being used in this study individually, and further investigate their beneficial effects on symptoms of depression in patients with MDD [15]. Also, probiotics interventions at early life with the focus on preventing future depression could become a research subject, as the gut microbiota shapes in early infancy and any interventions in later years need to continue for a long while, if not forever, to sustain the conferred benefit. Instituting a healthy gut in infancy and childhood, which would include vaccinating, preventing against mental disorders in later life, will be beneficial and more cost-effective, than taking psychobiotics during adulthood. Future research work in this new area of nutritional psychiatry, should focus on ensuring larger sample size and more sophisticated study designs, in order to establish the role of gut brain axis and afford sensitivity analyses to identify the predictors of treatment response. The leveling up of interventions and recognition of the pathways that regulate the effect of dietary improvement or probiotic supplementation on depressive illness are also key imperatives for future research models. Clinicians should also appraise highlighting the beneficial effects of dietary improvement and probiotic supplementation facilitating the access to dietetics support for their patients with depression [44].

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KEY TO MASTER CHART

RANDOMISATION CATEGORY	
A	PLACEBO
B	DRUG
AGE(YEARS)	
1	18-25
2	26-40
3	41-55
4	56-65
GENDER	
1	MALE
2	FEMALE
EDUCATION	
1	ILLITERATE
2	PRIMARY SCHOOL
3	MIDDLE SCHOOL
4	HIGH SCHOOL/ MATRICULATION
5	HIGHER SECONDARY/DIPLOMA
6	GRADUATE
7	HONOURS/POST GRADUATE
OCCUPATION	
1	SKILLED
2	SEMI SKILLED
3	STUDENT
4	FARMER
5	BUSINESSMAN/SELF EMPLOYED

6	UNEMPLOYED
7	HOMEMAKER
8	RETIRED
MONTHLY INCOME	
1	UPTO 10,000
2	10,000-20,000
3	20,000-30,000
4	30,000-50,000
5	>50,000
FAMILY TYPE	
1	NUCLEAR
2	EXTENDED
3	JOINT
SOCIOECONOMIC STATUS	
1	UPPER
2	UPPER MIDDLE
3	LOWER MIDDLE
4	UPPER LOWER
5	LOWER
MARITAL STATUS	
1	UNMARRIED
2	MARRIED
3	DIVORCED
4	WIDOWED
5	REMARRIED
FAMILY HISTORY OF PSYCHAITRIC ILLNESS	

1	YES
2	NO
H/O SUICIDAL IDEATION/ PLANNING/ BEHAVIOR AT WEEK 0	
1	YES
2	NO
PSY COMORBIDITY IF ANY ON M.I.N.I.	
1	NO
2	YES
COMPLIANCE TO ANTIDEPRESSANT (AT WEEK 3)	
1	YES
2	NO
COMPLIANCE TO ANTIDEPRESSANT(AT WEEK 6)	
1	YES
2	NO
COMPLIANCE TO DRUG/PLACEBO(AT WEEK 3)	
1	YES
2	NO
COMPLIANCE TO DRUG/PLACEBO(AT WEEK 6)	
1	YES
2	NO
CLASS OF ANTIDEPRESSANT PATIENT IS PRESCRIBED	
1	SSRI
2	SNRI

3	TCA
4	MAO INHIBITOR
5	OTHERS
6	MORE THAN ONE

SUBJECT S. NO.	MRD NO.	SUBJECT NAME	RANDOMISATION CATEGORY	AGE	SEX	EDUCATION	OCCUPATION	MONTHLY INCOME	FAMILY TYPE	SOCIOECONOMIC SATUS	MARITAL STATUS	FAMILY H/O PSY ILLNESS	H/O SUICIDAL IDEATION/ PLANNING/ BEHAVIOR AT WEEK 0	PSY COMORBIDITY IF ANY ON M.I.N.I.
1	925068	SR	A	3	2	3	7	1	2	4	2	1	1	1
2	44933	GS	B	3	1	3	4	1	2	4	2	2	1	1
3	936061	S	A	2	1	2	4	2	3	3	1	2	1	1
4	65906	MK	B	3	2	1	7	1	1	4	2	2	1	1
5	286964	BS	A	3	1	4	4	1	3	4	2	2	1	1
6	912042	NK	B	1	2	2	3	1	3	4	1	1	1	1
7	830102	JK	A	2	1	5	5	3	2	2	1	1	1	1
8	811848	PS	B	1	1	2	4	3	3	2	1	1	1	1
9	940504	KS	A	3	1	2	4	2	1	3	2	2	2	1
10	936606	TK	B	2	2	3	7	1	2	4	1	2	1	1
11	927905	RP	A	2	1	4	5	3	3	2	1	1	2	1
12	914128	MK	B	4	1	1	7	1	3	4	2	1	1	1
13	43966	RK	A	3	1	7	4	5	3	1	2	2	1	1
14	942725	AS	B	2	1	5	4	2	3	1	1	2	1	1
15	728133	BS	A	2	1	1	4	2	2	3	1	1	2	1
16	928910	DS	B	2	1	4	5	2	3	1	2	2	2	1
17	47569	KS	A	2	1	6	4	2	2	3	1	2	2	1
18	68503	HL	B	2	1	2	5	2	2	1	1	1	2	1
19	771669	RK	A	1	2	2	7	1	2	4	1	2	1	1
20	941735	PK	B	2	2	1	7	1	3	5	1	1	1	1
21	950490	HK	A	3	2	2	1	3	1	2	2	2	1	1
22	24053	AS	B	4	1	1	4	3	3	2	2	1	1	1
23	950998	SS	A	2	1	2	4	1	3	4	1	1	2	1
24	927320	K	B	2	2	1	7	1	2	4	2	1	2	1
25	376470	YS	A	3	1	7	5	2	3	1	2	2	2	1
26	532327	DN	B	3	1	1	2	1	3	4	2	2	2	1
27	47480	U	A	4	2	2	7	1	3	4	2	1	1	1
28	549578	SL	B	3	2	2	7	1	2	4	2	2	1	1
29	839637	R	A	1	1	6	3	1	1	4	1	1	1	1
30	958378	MK	A	3	2	1	7	1	3	4	2	2	1	1
31	358942	AR	B	4	2	1	7	1	3	4	2	1	1	1
32	406129	N	B	3	1	2	4	2	3	3	2	1	1	1
33	51126	JB	A	4	1	1	2	1	2	4	2	2	1	1
34	936892	HS	A	3	1	2	4	3	3	2	2	1	1	1
35	78991	AS	B	3	1	3	4	3	3	2	2	1	1	1
36	932927	PK	B	3	2	1	7	1	1	4	2	2	2	1
37	71641	SD	A	3	2	1	7	1	2	4	2	1	1	1
38	739009	SS	A	3	1	3	4	2	3	3	2	2	1	1
39	970657	AK	B	3	2	2	7	1	3	4	3	1	1	1
40	963288	SK	B	2	1	1	2	2	3	1	1	2	1	1
41	971161	MK	A	4	2	1	7	1	3	4	2	1	1	1
42	979921	VK	B	3	1	4	5	3	2	2	2	1	2	1
43	575411	JS	B	3	1	4	4	2	3	3	2	1	1	1
44	987633	AS	A	2	1	3	4	2	2	1	1	1	2	1
45	971159	SK	A	2	2	5	2	2	3	3	1	2	1	1
46	991140	JA	B	2	1	3	2	2	1	3	1	2	2	1
47	951519	N	A	3	2	5	7	1	2	4	2	1	2	1
48	979111	PC	B	2	2	5	5	1	2	4	1	2	1	1
49	991045	KK	A	2	2	4	2	2	3	3	1	2	2	1
50	255464	BS	B	3	1	6	5	4	3	1	2	1	1	1
51	925867	SS	A	1	1	5	3	1	1	4	1	2	1	1
52	984784	SK	B	2	2	4	7	1	3	4	2	2	1	1

SUBJECT S. NO.	MRD NO.	SCORE ON HAM-D			SCORE ON MADRS			COMPLIANCE TO ANTIDEPRESSANT		COMPLIANCE TO DRUG/PLACEBO		CLASS OF ANTIDEPRESSANT PT. IS PRESCRIBED
		WEEK 0	WEEK 3	WEEK 6	WEEK 0	WEEK 3	WEEK 6	AT WEEK 3	AT WEEK 6	AT WEEK 3	AT WEEK 6	
1	925068	34	26	24	50	46	39	1	1	1	1	1
2	44933	30	23	19	51	40	36	1	1	1	1	1
3	936061	35	30	27	51	47	42	1	1	1	1	1
4	65906	26	21	18	48	42	37	1	1	1	1	2
5	286964	19	15	13	27	24	19	1	1	1	1	1
6	912042	31	23	19	42	37	33	1	1	1	1	1
7	830102	30	27	23	49	46	39	1	1	1	1	1
8	811848	17	12	10	40	33	30	1	1	1	1	1
9	940504	16	9	4	26	14	7	1	1	1	1	2
10	936606	23	19	15	36	32	29	1	1	1	1	1
11	927905	31	27	26	44	40	33	1	1	1	1	1
12	914128	33	30	28	43	38	33	1	1	1	1	1
13	43966	30	26	24	50	46	40	1	1	1	1	1
14	942725	28	20	18	46	38	32	1	1	1	1	1
15	728133	18	15		31	25		1	2	1	2	1
16	928910	17	15	13	40	33	27	1	1	1	1	1
17	47569	19	12	5	32	20	8	1	1	1	1	1
18	68503	27	20	17	46	38	31	1	1	1	1	1
19	771669	31	28	26	48	46	30	1	1	1	1	1
20	941735	28	22	18	44	40	34	1	1	1	1	1
21	950490	38	22	8	48	32	12	1	1	1	1	1
22	24053	33	24	19	38	33	26	1	1	1	1	2
23	950998	29	26	23	43	41	34	1	1	1	1	1
24	927320	30	25	19	35	30	24	1	1	1	1	1
25	376470	27	23	21	41	37	32	1	1	1	1	1
26	532327	30	23	19	41	33	25	1	1	1	1	1
27	47480	27	24	22	41	38	32	1	1	1	1	1
28	549578	32	27	22	36	31	25	1	1	1	1	1
29	839637	32	29	26	48	46	41	1	1	1	1	1
30	958378	28	26	25	48	46	41	1	1	1	1	1
31	358942	30	28		50	46		1	2	1	2	1
32	406129	26	20	17	45	34	29	1	1	1	1	1
33	51126	21	13	5	30	19	8	1	1	1	1	2
34	936892	29	27	26	44	40	34	1	1	1	1	1
35	78991	22	19	17	42	35	29	1	1	1	1	1
36	932927	27	21	17	47	36	29	1	1	1	1	1
37	71641	29	26	24	48	46	40	1	1	1	1	1
38	739009	28	13	5	45	28	12	1	1	1	1	1
39	970657	29	24	19	40	34	30	1	1	1	1	1
40	963288	19	15	13	36	30	26	1	1	1	1	1
41	971161	27	24	22	40	37	30	1	1	1	1	1
42	979921	28	23	19	35	27	20	1	1	1	1	1
43	575411	31	25	20	44	35	28	1	1	1	1	1
44	987633	25	23	21	43	41	36	1	1	1	1	1
45	971159	30	26	25	42	40	35	1	1	1	1	1
46	991140	30	21	18	36	31	27	1	1	1	1	1
47	951519	29	25	23	39	36	31	1	1	1	1	1
48	979111	29	24	19	41	35	29	1	1	1	1	1
49	991045	24	22	21	38	35	29	1	1	1	1	1
50	255464	32	25	21	34	29	23	1	1	1	1	1
51	925867	30	27	25	40	38	34	1	1	1	1	1
52	984784	28	23	19	43	36	29	1	1	1	1	1

SUBJECT S. NO.	MRD NO.	SUBJECT NAME	RANDOMISATION CATEGORY	AGE	SEX	EDUCATION	OCCUPATION	MONTHLY INCOME	FAMILY TYPE	SOCIOECONOMIC SATUS	MARITAL STATUS	FAMILY H/O PSY ILLNESS	H/O SUICIDAL IDEATION/ PLANNING/ BEHAVIOR AT WEEK 0	PSY COMORBIDITY IF ANY ON M.I.N.I.
53	6054	BS	A	3	1	4	2	3	3	2	2	1	1	1
54	1024580	GK	B	2	2	2	3	1	3	4	1	2	1	1
55	1003994	AV	A	3	2	1	7	1	2	4	2	1	1	1
56	1024543	S	B	2	2	1	7	1	3	4	2	2	1	1
57	1024715	SB	A	1	2	3	3	1	1	4	1	1	2	1
58	63753	MR	B	2	2	2	7	1	2	4	2	1	2	1
59	1026811	ML	A	3	1	1	5	5	2	1	2	1	1	1
60	921448	JS	B	3	1	4	4	2	3	1	2	2	2	1
61	1027166	KS	A	4	1	2	4	5	3	1	2	1	1	1
62	278436	RS	B	2	2	7	4	5	3	1	2	2	1	1
63	42032	SK	A	3	2	5	2	2	3	3	2	2	1	1
64	278694	HS	B	2	1	1	6	1	1	5	3	1	1	1
65	38727	PV	A	4	2	4	2	1	3	4	2	1	1	1
66	1063852	RK	B	3	2	4	7	1	2	4	2	2	1	1
67	1052426	HS	A	1	1	6	4	5	3	1	2	1	1	1
68	1055685	S	B	2	2	3	7	1	1	4	1	2	1	1
69	1062381	H	A	2	2	6	1	4	2	1	1	2	1	1
70	1047438	HK	B	2	2	2	2	1	3	4	1	1	2	1
71	1049079	SS	A	2	2	1	1	4	2	1	2	2	1	1
72	1060914	HR	B	4	1	1	4	2	3	3	2	2	2	1
73	1080813	LS	A	2	1	2	6	1	3	4	1	2	1	1
74	1083345	JS	B	2	1	4	4	3	2	1	2	2	1	1
75	174673	NK	A	3	2	4	7	1	2	4	2	2	1	1
76	880726	AK	B	4	1	7	8	5	3	1	2	1	1	1
77	79094	J	A	3	1	4	4	2	3	1	2	1	2	1
78	1075956	JP	B	4	1	5	5	5	1	1	2	2	2	1
79	564746	SA	A	2	1	3	2	3	3	2	3	2	2	1
80	1007209	BK	B	2	2	5	5	2	2	3	1	2	2	1
81	459931	A	A	2	1	4	2	2	1	3	2	2	1	1
82	902555	GKV	B	2	1	2	5	4	3	1	5	2	1	1
83	1069545	AR	A	2	2	5	7	1	3	4	2	1	1	1
84	1086040	MK	B	2	2	5	2	2	2	3	2	2	1	1
85	41633	BK	A	3	2	2	7	1	3	4	2	2	1	1
86	559909	RS	B	2	1	3	2	3	3	2	2	2	1	1
87	349682	BK	A	4	2	1	7	1	3	4	2	1	2	1
88	74803	KS	B	2	1	5	4	3	3	2	2	1	2	1
89	1127321	AK	A	2	2	6	1	3	3	2	2	1	2	1
90	1127488	KG	B	2	2	5	1	4	2	1	3	2	2	1
91	398073	AP	A	2	2	1	7	1	3	4	1	2	1	1
92	1129050	RT	B	2	1	1	6	1	3	5	1	2	2	1
93	182731	RS	A	3	1	4	4	3	1	2	2	2	2	1
94	1142919	RK	B	2	2	3	2	2	3	3	2	1	1	1
95	506222	HL	A	3	1	2	4	1	2	4	2	1	2	1
96	60325	SS	B	3	1	4	1	4	2	1	2	1	1	1
97	254383	CM	A	3	1	2	5	2	3	3	2	2	2	1
98	1114271	KS	B	3	1	5	1	5	3	1	2	1	1	1
99	1143619	VS	A	2	1	2	2	2	3	3	2	1	1	1
100	1142759	AS	B	2	1	7	5	4	3	1	2	1	1	1

SUBJECT S. NO.	MRD NO.	SCORE ON HAM-D			SCORE ON MADRS			COMPLIANCE TO ANTIDEPRESSANT		COMPLIANCE TO DRUG/PLACEBO		CLASS OF ANTIDEPRESSANT PT. IS PRESCRIBED
		WEEK 0	WEEK 3	WEEK 6	WEEK 0	WEEK 3	WEEK 6	AT WEEK 3	AT WEEK 6	AT WEEK 3	AT WEEK 6	
53	6054	20	11	4	28	18	11	1	1	1	1	1
54	1024580	25	18	16	42	36	30	1	1	1	1	1
55	1003994	32	28	26	40	36	30	1	1	1	1	1
56	1024543	30	23	20	35	30	26	1	1	1	1	1
57	1024715	26	23	21	34	30	25	1	1	1	1	1
58	63753	24	19	18	44	33	28	1	1	1	1	1
59	1026811	30	25	23	48	46	39	1	1	1	1	1
60	921448	22	16	14	36	26	21	1	1	1	1	1
61	1027166	33	29	28	44	40	35	1	1	1	1	1
62	278436	29	19	16	40	35	29	1	1	1	1	1
63	42032	32	29	27	48	46	38	1	1	1	1	1
64	278694	34	29	28	48	45	39	1	1	1	1	1
65	38727	29	27	25	43	41	36	1	1	1	1	1
66	1063852	30	22	19	46	31	27	1	1	1	1	1
67	1052426	32	28	26	50	47	40	1	1	1	1	1
68	1055685	29	18	15	45	37	30	1	1	1	1	1
69	1062381	29	10	4	48	30	19	1	1	1	1	1
70	1047438	30	22	17	41	33	29	1	1	1	1	1
71	1049079	35	32	31	48	46	40	2	1	1	1	1
72	1060914	30	20	16	38	27	22	1	1	1	1	1
73	1080813	30	27	26	47	45	38	1	1	1	1	1
74	1083345	24	17	14	44	34	27	1	1	1	1	1
75	174673	29	26	24	45	41	36	1	1	1	1	1
76	880726	30	27	26	42	38	33	1	1	1	1	2
77	79094	19	11	4	27	20	10	1	1	1	1	1
78	1075956	20	14	11	45	35	26	1	1	1	1	1
79	564746	30	28	27	44	43	33	1	1	1	1	1
80	1007209	29	22	18	37	30	24	1	1	1	1	1
81	459931	21	19	18	29	26	21	1	1	1	1	1
82	902555	28	19	16	49	39	33	1	1	1	1	1
83	1069545	32	30	28	44	42	36	1	1	1	1	1
84	1086040	33	22	19	46	34	28	1	1	1	1	1
85	41633	29	27	26	40	38	35	1	1	1	1	1
86	559909	32	24	20	45	35	26	1	1	1	1	1
87	349682	26	25	23	36	34	29	1	1	1	1	1
88	74803	31	22	18	40	32	25	1	1	1	1	1
89	1127321	29	27	25	45	43	38	1	1	1	1	1
90	1127488	30	24	20	41	34	27	1	1	1	1	1
91	398073	33	31	29	48	47	42	1	1	1	1	1
92	1129050	33	24	19	41	37	30	1	1	1	1	1
93	182731	26	24	23	41	39	34	1	1	1	1	1
94	1142919	29	26		35	31		1	2	1	2	1
95	506222	25	14	6	35	22	10	1	1	1	1	2
96	60325	28	16	13	36	35	30	1	1	1	1	1
97	254383	32	30	29	45	44	38	1	1	1	1	1
98	1114271	28	21	18	35	35	28	1	1	1	1	1
99	1143619	25	23	21	35	33	30	1	1	1	1	1
100	1142759	28	24	22	37	30	24	1	1	1	1	1

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Date 20/4/2021

The Principal,
DMC,Ludhiana.

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 22.01.2021 has approved the plan of thesis of Dr. Deepika Bansal, a student of your college for MD Psychiatry along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Probiotics as an add-on in major depressive disorder

Supervisor
Dr. Ranjive Mahajan
Prof. & Head,
Dept. of Psychiatry,
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Co-Supervisor
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The student has to submit the three copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

1. ***If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.***
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

Doctor of Medicine
in

PSYCHIATRY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES
FARIDKOT

NOVEMBER, 2019

DR. DEEPIKA BANSAL

DEPARTMENT OF PSYCHIATRY
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	'PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER'
For the degree of	MD PSYCHIATRY
Name of the candidate	Dr. Deepika Bansal
Supervisor	Dr. Ranjive Mahajan, Professor and Head Department of Psychiatry
Co-supervisor	Dr. Navkiran Sooch Mahajan, Professor, Department of Psychiatry Dr. Sandeep Kaushal, Professor and Head, Department of Pharmacology
Institution	Dayanand Medical College & Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Individuals experiencing major depressive disorder bear high levels of illness burden and a significantly reduced quality of life. Despite the available psychopharmacological strategies and complementary psychotherapeutic procedures the desired results are not seen, and the relapse rate is more. Major depressive disorder is a result of multifactorial pathogenesis consisting of genetics, inflammation and neurotransmitter imbalance as well as lifestyle-associated factors. In the past decade, research on the gut-brain axis has grown tremendously showing connection between gut microbiota and psychiatric disorders. The evidence of gut microbiota having an impact on the onset and course of depression is the area of focus showing that probiotic supplementation as an adjuvant therapy in patients of major depressive disorder shows a rapid improvement in alleviating the symptoms of depression.

Aim of Study: To study the effect of probiotic supplementation on symptoms of depression in patients of major depressive disorder already on antidepressants.

Materials & methods: The study will be conducted on patients(N=100) of Major Depressive Disorder presenting to DMC&H. The patients will be randomly assigned to receive either probiotic or the placebo for 6 weeks and psychiatric evaluation will be done using Mini International Neuropsychiatric Interview (M.I.N.I.), Hamilton Depression Rating Scale(HAM-D), Montgomery-Asberg Depression Rating Scale(MADRS).

Clinical Significance: This study will help us in understanding the relationship between gut microbiome and depression and will show the response of treatment in patients of depression when supplemented with probiotics.

Keywords: Gut-Brain Axis, Major Depressive Disorder, Gut Microbiome, Probiotics, Psychobiotics, Dietary Patterns, Randomized control trail

(Signature) Candidate	(Signature) Co-supervisor	(Signature) Co-supervisor	(Signature) Supervisor	(Signature) Principal
Dr DEEPIKA BANSAL	Dr. NAVKIRAN SOOCH MAHAJAN	Dr. SANDEEP KAUSHAL	Dr. RANJIVE MAHAJAN	Dr SANDEEP PURI

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER"** exist in the Department of Psychiatry, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate himself under our supervision and guidance.

Supervisor

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APPROVAL PROFORMA
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Name of candidate	Dr. Deepika Bansal
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Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Jaspal Singh (Professor & Head, Surgery)	Mr. A K Jindal (Member, Ethics Committee)
Dr. Dinesh Gupta (Professor & Head, Medicine)	Dr. Anu Sharma (Professor, Anatomy)
Dr. Sandeep Kaushal (Assistant Dean Academics)	Dr. Deepinder K Chhina (Professor & Head, Microbiology)
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	Dr. Gagandeep Singh (Secretary, Ethics Committee Professor & Head, Neurology)

Approved: Yes / ~~No~~

Dr. Sandeep Puri
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Approved: Yes / ~~No~~

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Chairperson
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BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

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2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE AND HOSPITAL, LUDHIANA (PUNJAB)
3	COURSE OF STUDY AND SUBJECT	M.D PSYCHIATRY
4	DATE OF ADMISSION OF COURSE	18.05.2019
5	TITLE OF THE TOPIC	"PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER"

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Major Depressive Disorder (MDD) is a complex and multifactorial disorder that involves marked disabilities in global functioning, anorexia and severe medical comorbidities. It affects around 20% of the population at some point during their lifetime.[1] MDD is a devastating yet widespread chronic disorder with considerable personal and socioeconomic costs. According to World Health Organization, MDD is the fourth leading cause of disability worldwide, and it will be the second leading cause by 2020. Individuals suffering from MDD experience persistently low mood, blunted or absent experience of pleasure, as well as wide range of cognitive, physical and behavioral symptoms that interfere with the individuals capacity to function in daily life. First line pharmacological treatments for MDD have an increasingly high failure rate, thus identifying new treatment targets and therapies is very important. Previously, it was generally accepted among clinicians that MDD was a disorder of reduced monoaminergic activity in the brain. Consequently most pharmacological treatments currently available solely target those neurotransmitters and their receptors. Others have also postulated involvement of glutamate receptor; NMDA(N- Methyl D-Aspartic acid) , or the cholinergic or GABAergic(gamma amino butyric acid) transmission[2]

There is now a great body of evidence that indicate that pathophysiological pathways involved in the pathogenesis of depression are influenced by disturbance in the equilibrium of the gut microbiota[3].The intestine and the brain are intimately connected via the gut-brain axis, which involves bidirectional communication via neural, endocrine and immune pathways. In recent years it has become increasingly evident that this communication also involves interactions with the intestinal microbiota, which release immune activating and other signaling molecules that may play an important role in regulating the brain and subsequent behavior[4]. There is emerging evidence from preclinical studies of role for gut microbiota on the central nervous system function[5].

Currently, most pharmacological treatments target neurotransmitter activity in the brain and are known to have a delayed onset of effect and a range of side effects. Further it has been estimated that up to 60% of patients with MDD experience some degree of non response to these treatments[6].

Driven by rapid urbanization, our lifestyle and food changed drastically in the last decades. These changes are related to the high prevalence of depression and other psychiatric disorders in western, urbanized countries. In line with this, diet and nutrition are viewed as an important factor contributing to the pathogenesis of somatic as well as psychiatric disorders[7]. Numerous pathways between the gut and brain have been delineated, and the data indicate that gut-brain communication is bidirectional and mediated by neural and humoral mechanisms. With neural pathways acting in a rapid and somatotropic manner combined with the slower and somatically less specifc humoral route, these complementary pathways facilitate the effects of gut microbiota on brain and behavior[8]. The two key subcortical structures that generate these outputs are the hypothalamus and the amygdala[9].

Probiotics are currently defined as a live organism that when ingested in adequate amounts, exert a health benefit[10]. In this respect, recent studies in animals have indicated that gut microbiota transplantation can transfer a behavioral phenotype, suggesting that the gut microbiota may be a modifiable factor modulating the development or pathogenesis of neuropsychiatric conditions[11]. Several studies, mostly preclinical or a limited group of small clinical studies, have shown that prebiotic and probiotic ingestion in adults can modulate brain function and behavior, an observation that has led to the term psychobiotics[12]. There is growing interest in the role of the intestinal microbiota in health and disease. Gut bacteria not only instruct and shape the host immune system, and impact its metabolism, but also affect function in the gut and central nervous system[13]. The term 'psychobiotics' is now being used to address these agents in psychiatric research[14]. There is abundant evidence demonstrating that Lactobacillus and Bifidobacterium attenuated anxiety, prevented the chronic psychological stress, reduced

apoptosis in several brain regions, and improved learning and memory in mice. Thus, it proposed that gut microbiota could be associated with brain function as well as neurological diseases via the gut-brain axis[15]. Although the e□ect of gut dysbiosis on host health has received special attention in recent years, studies on the relationship between GI track health and CNS function, especially depression, are rare. We designed the present study to assess the effects of synbiotic supplementation in the treatment of moderate depression in a double- blind, placebo-controlled, randomized trial during 6weeks[16]. Probiotic bacteria not only modulate host immune responses, but also create a healthy gut environment through balancing of the intestinal microflora. Ingestion of probiotics may restore the composition of the gut microflora to a state more favorable for beneficial microorganisms[17].

Evidence now suggests that the bacterial commensals of the gut can influence conditions such as depression, schizophrenia and autism[18]. This study is an important contribution to the understanding of the effect of therapeutic modulation of gut microbiota using specific strains of probiotics on brain function[19]. More recently, 30-day consumption of a probiotic mixture containing Lactobacillus helveticus and Bifidobacterium longum showed beneficial effects on anxiety and depressive measures and showed reduced levels of stress hormone, cortisol, in healthy volunteers[20]. Current evidence suggests that multiple mechanisms, including endocrine and neurocrine pathways, may be involved in gut microbiota–to–brain signaling and that the brain can in turn alter microbial composition and behavior via the autonomic nervous system[21]. This proposal seeks to build on prior research to produce an inclusive whole food-based nutrition intervention that potentially will have a more significant impact on depressive disorders. This research will be combining well studied probiotics with a beneficial dietary pattern in the hopes of creating a protocol that yields a more pronounced reduction on depressive symptoms, which could be used in a clinical trial or as an adjuvant treatment[22].

6.2 REVIEW OF LITERATURE

Depression is one of the most common mental disorders globally and a leading cause of disability affecting more than 300 million people worldwide (Kessler & Bromet, 2013; World Health Organization, 2018). Depression greatly reduces quality of life and can have major personal, professional, and social consequences. It also has the capability to bring on suicidal thoughts and potentially lead to death (Popa & Ladea, 2012). According to the Diagnostic and

Statistical Manual of Mental Disorders (5th ed.), the myriad of depressive disorders share common diagnostic criteria including: "the presence of sad, empty, or irritable mood, accompanied by somatic and cognitive changes that significantly affect the individual's capacity to function" (American Psychiatric Association, 2013)[22].

A study from Iran by Akkasheh et al carried out a randomized control, double blinded, placebo controlled trial including 40 patients with a diagnosis of MDD and it was observed that probiotic administration in patients with MDD for 8 weeks had beneficial effects on Beck Depression Inventory. The administration of probiotics might result in improvement of symptoms of depression through increased plasma tryptophan levels, decreased serotonin metabolite concentrations in pre frontal cortex, and decrease dopamine metabolite concentration in amygdaloid cortex. Various factors including host physiology, immunology, diet, antibiotic use, and enteric infection can affect the gut microbiota composition and its activity. Probiotic bacteria through fermenting dietary ingredients might lead to specific changes in composition or activity of gastrointestinal microbiota, which might result in improved peripheral (gastrointestinal) and central(psychological) symptoms.[1]

Probiotics are currently defined as a living organism that, when ingested in adequate amounts, exerts a health benefit. A new term emerging nowadays, "Psychobiotics" is defined as a living organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness. Logan and Katzman first proposed the use of probiotics as adjunct therapy in the management of depres-

sion. Certain strains of Lactobacillus and Bifidobacterium secrete gamma-amino butyric acid which is the main inhibitory neurotransmitter in the brain regulating many physiological and psychological processes, with dysfunction in the system implicated in anxiety and depression[10].

Effective first-line antidepressant medications are currently limited. Many patients try 2–3 different formulations or combinations before achieving symptom relief, delaying the onset of possible recovery. Even then, up to 60% of patients discontinue their antidepressant use within 3 months. One of the most common reasons for this is side effects, which can include nausea, vomiting, headache, agitation or sedation, sexual dysfunction, and weight gain. In 2013, a study by Bet et al. showed that over two-thirds of patients experience up to 3 side effects that often persist throughout long-term antidepressant use. Moreover, many individuals do not seek treatment for their depression at all due to the stigma associated with mental illness and antidepressant medication, preventing recovery and perpetuating the disease cycle. Probiotic supplementation is considered low risk and may be a favorable alternative to classical antidepressants due to the absence of side effects or adverse reactions noted in our pilot research. Furthermore, probiotic supplementation does not carry with it the stigma that antidepressant medications do, an issue that can be a barrier to seeking treatment. Finally, probiotic supplementation may even lead to improvements in overall general health[2].

The adult human GI tract is the natural habitat of a large and dynamic population of micro-organisms thriving in the relationship between external and internal environments. It comprises at least 160 of different bacterial species per individual from the pool of 1000 and 1150 prevalent species of bacteria, among which probiotics administered in adequate amounts are proposed to confer a health benefit and as a novel therapeutic strategy, particularly for mood disorders. One probiotic formulation (PF), a combination of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175, showed beneficial effects on GI symptoms in patients subjected to chronic stress. When administered sepa-

rately, these two strains have also showed beneficial effects. For example, several strains of Lactobacillus displayed anti-inflammatory properties in vitro in human intestinal epithelial cells, while oral treatment with B. longum R0175 showed beneficial properties in human subjects with ulcerative colitis. Moreover, L. helveticus had favourable actions on sleep efficiency in elderly subjects. The role of inflammatory processes on emotion is indicated by findings of a link between depression and elevated levels of IL-6, TNF and C-reactive protein. Systemically injected cytokines induce depressive symptoms, prevented by antidepressants. It has been suggested that antidepressants act in part via generation of perhaps the most potent immunoregulatory cytokine, IL-10, thereby suppressing inflammation and depressive mood. Lactobacillus and Bifidobacterium strains attenuated inflammatory responses or else induced IL-10 production in rodents. In accordance with this finding, both L. helveticus R0052 and B. longum R0175 showed anti-inflammatory properties in human cell lines. Thus, bacteria may be used to influence mood in patients with elevated inflammatory chemicals. [5]

In a study by Department of clinical nutrition, Iran by Kazemi et al, a double blinded clinical trial including 110 clinically depressed patients showed that probiotic supplementation for 8 weeks resulted in significant decrease in Beck Depression Inventory Scale [3].

A study from Netherlands carried out triple blinded, placebo controlled, randomized control trial, carried out in 20 healthy participants showed that a 4 week multispecies probiotic intervention showed a significantly reduced overall cognitive reactivity to sad mood[4].

The concept that the gut and the brain are closely connected, and that this interaction plays an important part not only in gastrointestinal function but also in certain feeling states and in intuitive decision making, is deeply rooted in our language. Recent neurobiological insights into this gut–brain crosstalk have revealed a complex, bidirectional communication system that not only ensures the proper maintenance of gastrointestinal homeostasis and digestion but is likely to have multiple effects

on affect, motivation and higher cognitive functions, including intuitive decision making.[9]

Currently it is well accepted that our gut microbiota is critical for brain processes such as myelination, neurogenesis and microglial activation and can effectively modulate behavior and influence psychological processes such as mood and cognition. Indeed, very recently gut microbiota have been shown essential for the maintenance of microglia in a healthy functional state, which is necessary for the prevention of neurodevelopmental and neurodegenerative disorders. Nowadays it is clear that certain bacteria are strain-specifically able to produce different essential neurotransmitters and specific neuromodulators. Indeed, several neurotransmitters such as gamma-aminobutyric acid (GABA), serotonin, catecholamines and acetylcholine are produced by bacteria, some of which are inhabitants of the human gut. Indeed, researchers report that Lactobacillus spp. and Bifidobacterium spp. produce GABA; Escherichia spp., Bacillus spp. and Saccharomyces spp. produce noradrenalin; Candida spp., Streptococcus spp., Escherichia spp. and Enterococcus spp. produce serotonin; Bacillus spp. produce dopamine; and Lactobacillus spp. produce acetylcholine. Neurotransmitters secreted from bacteria in the intestinal lumen may induce epithelial cells to release molecules that, in turn, have the ability to modulate neural signaling within the enteric nervous system
and subsequently control brain function and behavior.[11]

In a study in Iran, assessment of the e□cacy of 6 weeks probiotic supplementation in the treatment of moderate depression in a double-blind, placebo-controlled, multi-center, and randomized trial was done. There was reduction in HAM-D scores at end of 6 weeks in depressive patients.[16]

6.3 AIMS AND OBJECTIVES OF THE STUDY

To study the effect of probiotic supplementation on symptoms of depression in patients of major depressive disorder already on antidepressants.

7. MATERIALS AND METHODS

7.1 Source of Data

Study will be conducted on recently diagnosed 100 patients in age group of 18-65 years already on treatment for depression for a period of 6-12 weeks diagnosed as Major Depressive Disorder presenting to Department of Psychiatry in DMC&H, Ludhiana.

7.2 METHOD OF COLLECTION OF DATA

SAMPLE COLLECTION- Study will be conducted on recently diagnosed 100 patients in age group of 18-65 years already on treatment for depression for a period of 6-12 weeks diagnosed as Major Depressive Disorder presenting to Department of Psychiatry in DMC&H, Ludhiana. The patients will be randomly allocated into 2 groups of 50 each receiving either the probiotic or the placebo.

INCLUSION CRITERIA:

1. The patients with a diagnosis of Major Depressive Disorder according to DSM-5 criteria presenting to Department of Psychiatry in DMC&H, Ludhiana.
2. Age group between 18-65 years.
3. Patients who have agreed upon no changes in their routine physical activity and dietary intake throughout the study.

EXCLUSION CRITERIA:

1. Any other major psychiatric disorder in patients of Major Depressive Disorder.
2. Regular intake of probiotics during last 2 months before recruitment for the study.
3. History of any renal, hepatic, cardiovascular or respiratory disease in the patient.
4. Intake of antioxidant or omega 3 supplements less than 6weeks before beginning of study.
5. Female patients currently having pregnancy and lactation.
6. Any type of substance abuse in the patient.
7. Patients with history of heart attack and stroke following a specific diet.

8. Patients with intake of antibiotics 2 weeks before the study or during study.
9. Immunocompromised patients.
10. Patients who have not given consent for the study.

METHODOLOGY

The study will include recently diagnosed 100 patients in the age group of 18-65 years presenting with Major Depressive Disorder to the Department Of Psychiatry in DMC&H, Ludhiana. Those patients who have received treatment of depression for 6-12 weeks will be considered. The patients will be randomly allocated into two groups of 50 each receiving either the probiotic or the placebo. The patients will be screened using MINI scale having only MDD and severity of depression will be assessed at 0,3,6 weeks each using HAM-D and MADRS scales. To be eligible, patients had to meet the inclusion criteria and provide written informed consent before enrolment.

STUDY DESIGN: The study is a randomised, double blinded, placebo controlled, post-intervention assessment study to be carried out in 100 patients. Patients meeting the inclusion criteria will be randomly allocated into two groups to receive either probiotic supplement (n=50) or the placebo(n=50) for 6 weeks. Patients in probiotic group will receive daily one probiotic capsule, containing probiotic not less than 2.5 billion cells of *Lactobacillus acidophilus*(R 0052), *Lactobacillus rhamnosus* (R 0011), *Bifidobacterium longum* (R 00175), *Bacillus coagulans*(SNZ 1969), and Saccharomyces boulardii. Subjects in the placebo group will receive the placebo that contains starch but no bacteria. The appearance of the placebo is indistinguishable in color, shape, size, packaging, smell and taste from that of the probiotic supplement. Randomization will be done using computer generated random numbers. The clinical response to probiotic administration in depressive patients will be assessed by Mini International Neuropsychiatric Interview (M.I.N.I.), Hamilton Depression Rating Scale(HAM-D), Montgomery-Asberg Depression Rating Scale(MADRS).

TOOLS:

1. **Mini International Neuropsychiatric Interview-** MINI questionnaire for the assessment of the psychiatric co-morbidities. The Mini-International Neuropsychiatric Interview (M.I.N.I.) version 5.0.0 is a short structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and Europe, for psychiatric disorders. It is divided into 16 modules-identified by letters, each corresponding to a diagnostic category. With an administration time of approximately 15 minutes, it was designed to meet the need for a short but accurate structured psychiatric interview for multi-centre clinical trials.

2.Hamilton Rating Scale for Depression(HAM-D)- It is a multiple item questionnaire used to provide an indication of depression, and as a guide to evaluate recovery. Max Hamilton originally published the scale in 1960[3] and revised it in 1966, 1967, 1969, and 1980. The questionnaire is designed for adults and is used to rate the severity of their depression by probing mood, feelings of guilt, suicide ideation, insomnia, agitation or retardation, anxiety, sedation and somatic symptoms. HAM-D is a 21 multiple choice questionnaire which doctors may use to rate the severity of depression in a patient. Each question has 3-5 possible responses which increase in severity . Interpretation of score is as follows-

- **0-9**- normal
- **10-13**- mild depression
- **14-17**- moderate depression
- **>17**- severe depression

3. **Montgomery Asberg Depression Rating Scale(MADRS).**

The Montgomery- Asberg Depression Rating scale is a 10 item diagnostic questionnaire used to measure the severity of depressive episodes in patients with mood disorders. It was designed in 1979 by British and Swedish researchers. The items are rated on a continuum of 0-6.The questionnaire includes questions on following symptoms – Apparent sadness, Reported sadness, Inner tension, Reduced sleep, Re-

duced appetite, Concentration difficulties, Lassitude, Inability to feel, Pessimistic thoughts, Suicidal thoughts

The interpretation of scale is as follows-

- **0-6**- Normal/ Symptoms absent
- **7-19**- Mild Depression
- **20-34**- Moderate Depression
- **>34**- Severe Depression

OUTCOME

In this study, the primary outcome will be assessed on Mini International Neuropsychiatric Interview (M.I.N.I.), Hamilton Depression Rating Scale (HAM-D), Montgomery Asberg Depression Rating Scale(MADRS). Depressed mood will be judged in the beginning, after 3 weeks and at the end of study and the scores will be compared. The present study will draw attention to the fact that the use of probiotics in depressive patients along with the anti depressant therapy might prove beneficial for the early recovery of symptoms in these patients.

Statistical Analysis

Data will be described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups will be done using Student t-test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and exact test was used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

YES.

The patients will be prescribed one capsule of probiotic, SPOROLAC PLUS, or the placebo, daily for a period of 6 weeks. The medication as well as the placebo will be provided by the Sanzyme Pharmaceuticals.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Attached herewith

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SOCIO – DEMOGRAPHIC PROFORMA

DATE-

1.S.No.

2.Randomization No.

3.Mrd No.

4.Name

5.Age a)18-25 years b)26-40 years c)41-55 years
d)56-65 years

6.Sex a)Male b)Female

7.Address

8.Education: a) Illiterate b)Primary c) Middle
d)Matriculation d) Higher Secondary/Diploma
e) Graduate/ Post Graduate

8.Occupation: a)Skilled b) Semi Skilled c)Student d) Farmer
e) Self employed f)Unemployed g)Homemaker i) Retired

9.Monthly Income: a) Upto 10,000 b)10,000-20,000
c)20,000-30,000 d)30,000-50,000 e)>50,000

10.Family Type: a) Nuclear b) Extended c) Joint d) Other

11.Marital Status: a) Unmarried b) Married c) Divorced d) Widowed
e) Remarried

13.Family H/O Psychiatric Illness: a) Yes b) No

Signature of Candidate **Signature of Supervisor**

14.H/O Suicidal ideations/planning/behaviour By Patient (at 0 weeks)

a) Yes b) No

15. Psychiatric comorbidity if any (ASSESSED BY M.I.N.I.)-

16.Score on HAM-D at - 0 week
- 3 week
- 6 week

17.Score on MADRS at -0 week
-3 week
-6 week

18.Class of Anti depressants pt. has been prescribed-

a) SSRI b) SNRI c) TCA d) Mao inhibitor e) others
f) More than one

19. Compliance to anti depressant:

At 3 weeks	a) Yes	b) No
At 6 weeks	a)Yes	b)No

20. Compliance to drug used(probiotic/placebo):

At 3 weeks	a) Yes	b) No
At 6 weeks	a)Yes	b)No

21.CONTACT NO.

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o.....
.....am willing to participate in the study **"PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

.....
Signature of the Witness

.....
Signature of the patient

Name:

Signature of Candidate

Signature of Supervisor

फार्म अनुमति मरीज

तिथि_____

मैं_____ पत्नी/पुत्र/ पुत्री_____

निवासी_____ इस

शोधकार्य **"PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER"** लिए के लेने भाग में शोधकार्य इस मैं हूँ। सहमत लिए के लेने भाग में करता पुष्टि में हूँ। तैयार से स्वेच्छा अपनी/ लिए के देने स्वीकृति यह की हूँ करती इस मुझे है। गया डाला दबाव ही न और गया दिया नहीं प्रलोभन आर्थिक कोई मुझे यह की है सविश्वा यह मुझे और है गया दिया कर स्पष्ट श्यउद्दे का शोधकार्य के जोखिम वाले होने में शोधकार्य इस मुझे है। लिए के लाभ के मानवता और विज्ञान है। मामुली बहुत जोखिम यह में समझ मेरी और है गया दिया समझा में बारे

अपनी समय भी किसी में सम्बन्ध के लेने भाग में र्यशोधका इस मैं सकता कर व्यक्त अस्वीकृति/ मेरे का करने व्यक्त अस्वीकृति मेरे और हूँ सकती पड़ेगा। नहीं प्रभाव कोई पर इलाज

_____ _____

हस्ताक्षर के गवाह हस्ताक्षर के रोगी/ निशान का अंगूठे

नाम: नाम:

_____

अन्वेषक केहस्ताक्षर

_____

हस्ताक्षर के विद्यार्थी

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"PROBIOTICS AS AN ADD-ON IN MAJOR DEPRESSIVE DISORDER" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ